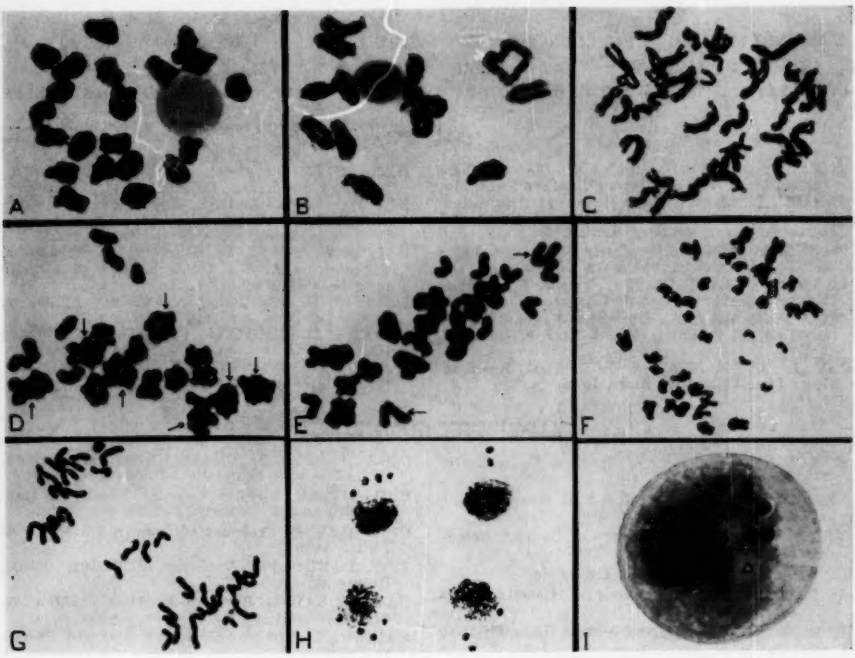
The Journal of HEREDITY



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Sex Control in Cucumbers

Cattle With Double Cervices

A Spontaneous Mosaic in Drosophila

Inheritance of Diapause in Grasshopper Eggs

VOLUME 52 JANUARY-FEBRUARY, 1961 NUMBER 1

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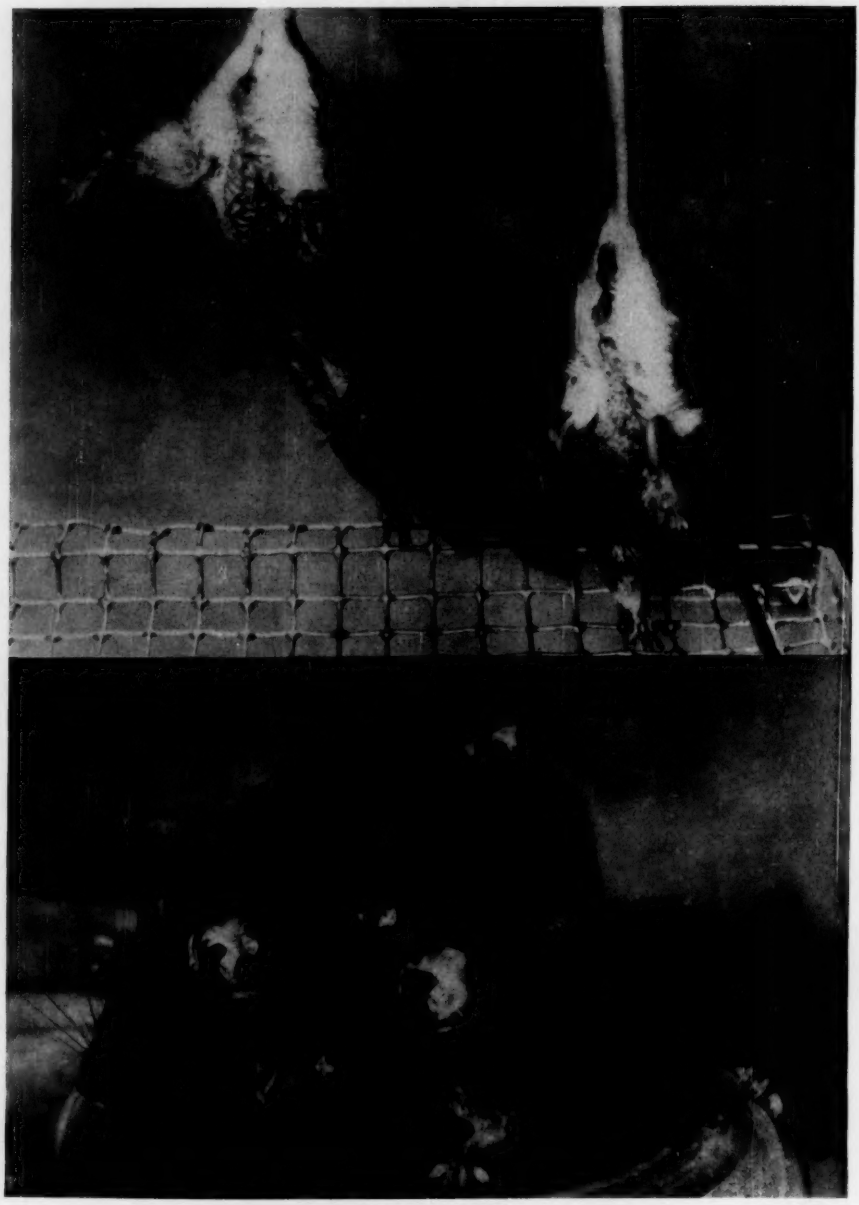


"LIGHT" MICE

Figure 1

A C57BL mouse is compared ventrally and dorsally with two "light" mice, one by C57BL/10 ♀2400, the other by C57BL/6J ♀6042. In the "light" mice, the belly is almost white, though flecked with black hairs, and the back is predominantly black, though flecked with white hairs.

NON-HERITABLE COAT COLOR VARIATION

A Multiple Recurrence in the House Mouse

G. D. SNELL, PRISCILLA W. LANE AND PAULINE HARNISH*

DICKIE[1] has described the appearance in a single mouse of a non-heritable dilution of the coat color suggestive of the Himalayan dilution in the rabbit. Since the mouse was heterozygous for albinism, an inversion of the dominance of *C* over *c* may possibly have been involved[2]. This paper describes multiple recurrences of a striking but non-heritable coat color variation in mice of the C57BL strain.

A C57BL/10ScSn female, number 2400, was mated in succession to two different males. One, number 221, was 31/32 C57BL/10 ancestry and was heterozygous for albinism (*c*); the other, number 2886, was pure C57BL/10. By male 221 she produced two litters. The first consisted of eight young at weaning, all normal; the second of four males and two females at weaning, all with an identical coat color dilution (Figure 1). The belly was almost white, though flecked with black hairs, and the back was predominantly black, though flecked with white hairs, particularly in the underfur which appeared quite light when the hair was blown back. Two subsequent litters by male 2886, totaling 11 young, all showed the same coat color variation. For convenience, we shall refer to this variation as "light."

About six months later, in a different animal room, a pair of C57BL/6J produced a litter of five with what was to all appearances the identical coat color deviation. Several previous litters by the same pair had all been normal. One subsequent litter contained one normal mouse and one mouse with a partly lightened coat. This was the only deviant animal out of 23 which did not conform to a strictly uniform type.

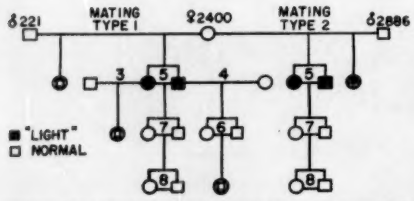
Since in each of these separate occurrences, the unusual coat color had all the appearances of a typical mutation, a breeding program was instituted, but in neither instance was the variant type recovered in later generations. The matings made and the descendants reared

TABLE I. Breeding tests of C57BL/10 female 2400 and her progeny

Mating type number*	Mating type	Number of pairs mated	Number of litters	Number of offspring classified	Number "light"
1	♀ 2400 × ♂ 221	1	2	14	6†
2	♀ 2400 × ♂ 2886	1	2	11	11
3	♀ light × ♂ normal	1	1	4	0
4	♀ normal × ♂ light	3	7	34	0
5	light × light	2	7	41 (+ 1*cc*)	0
6	$F_1 \times F_1$	4	17	86 (+ 7*cc*)	0
7		17	47	215 (+ 5*cc*)	0
8		7	19	100	0

*The mating type numbers correspond to those indicated in Figure 2.
†All in the second litter.

*Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. This study was supported by research grant C-1329 from the National Cancer Institute of the National Institutes of Health, Public Health Service, and by grant E-162 from the American Cancer Society.



TEST MATINGS WITH C57BL/10 ♀2400

Figure 2

The numbers of animals raised are not shown in this pedigree, but are indicated in Table I.

in the case of C57BL/ female 2400 are indicated in Figure 2 and Table I. The tests with the variants in strain C57BL/6 were: matings of lights *inter se* (54 young, all normal); F_1 from the cross light × C57BL/6J (23 young, all normal); F_2 from this cross (60 young, all normal); backcross to light (71 young, all normal).

It is clear that the character is not inherited as an ordinary dominant or recessive, either sex-linked or autosomal. The possibility that it depended on the genotype of the mother rather than on that of the affected individuals themselves was considered. Female 2400 might have produced light young because she was homozygous for a recessive gene, though the young themselves were heterozygous. This possibility appears to be ruled out by matings of types 7 and 8 (Figure 2), since the postulated genotype of female 2400 should have recurred among the 24 females used in these matings. Though albinism was carried by one of the males used, it cannot have been a factor since it was absent in other crosses that produced light individuals. An hereditary component may have been involved in the production of the trait, since in both instances it occurred in C57BL sublines, but it evidently is not hereditary in the ordinary sense.

Summary

Two females in each of two different C57BL sublines produced 23 young with an unusual lightening or silvering of the coat. Other young produced by the same females were normal. Extensive breeding tests failed to result in a reappearance of the deviant coat color in subsequent generations.

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1. DICKIE, M. M. A unique "Himalayan" mouse. *Jour. Hered.* 36:264-265. 1945.

2. GRÜNEBERG, H. The Genetics of the Mouse. Second edition. The Hague. Martinus Nijhoff. 1952.

SEX CONTROL IN CUCUMBERS

OVED SHIFRISS*

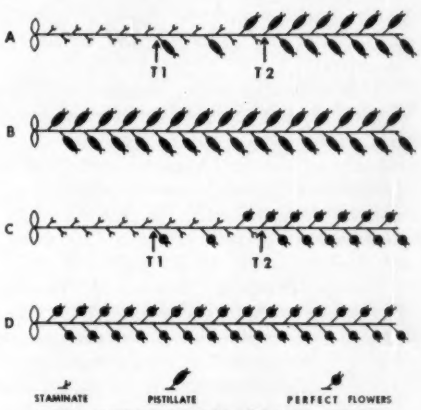
THE cultivated species of cucumbers, *Cucumis sativus* L., is predominantly monoecious but it consists of quantitative sex variations, ranging from almost male to completely female individuals. The objective of the present paper is to discuss some data bearing upon the developmental aspect of sex control in this crop.

Sex Variations

The standard varieties differentiate flower buds at all leaf axils under both short and long day conditions. As development proceeds, there is a gradual increase in growth rate of flower buds, in female tendency, and in ability of fruits to set parthenocarpically. This gradient course of reproductive behavior is typical of several of our cultivated cucurbits[7].

Four basic sex forms can be recognized: monoecism, gynoecism, andromonoecism, and hermaphroditism (Figure 3). Genetic males are not known in this species.

The gradient course of sex differentiation in monoecious varieties consists of strictly male, mixed, and strictly female phases in a progressive order. This gradient is particularly striking along the main stem (Figure 3*A*). The branches always reach the terminal phase sooner than the main stem and some branches are entirely female. Monoecious varieties differ from each other in sex tendency, i.e., in the proportion of staminate to pistillate flowers. Male tendency may be measured by the percent of staminate flowers and female tendency by the percent of pistillate flowers. However, the percent of either kind of flower cannot be employed effectively in genetic studies because it varies in time and location during plant development. A fairly reliable and useful measure of sex tendency in some varieties is the average number of leaves from the cotyledons to the first turning point, T1, on the main stem (Figure 3*A*)[14]. The higher the T1 is, the stronger the male tendency.



SEX EXPRESSION ALONG THE MAIN STEM

Figure 3

A—Monoecist. *B*—Gynoecist. *C*—Andromonoecist. *D*—Hermaphrodite. Flowers are differentiated at each leaf axil from the cotyledons onward. The leaf numbers to the turning points, T1 and T2, give reliable estimates of sex tendency.

The source of gynoecism (Figure 3*B*) is a group of Japanese[3, 17] and Korean[4, 6] races, e.g., Shoigon, Kurume, Seoul Mady, Black Pearl, and Chung Yup. These races include gynoecists and monoecists in varying proportions. Tkachenko[17] reported that "femaleness" and "maleness" in one of the Japanese varieties are governed by a pair of genes, "femaleness" being dominant. The results of experiments initiated by the writer in 1950 have shown that the expression of the factor associated with gynoecism varies considerably in different crosses and under different ecological conditions[1, 12]. This variation affects male tendency and is manifested as

*Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University, Department of Horticulture, New Brunswick. The writer wishes to thank Dr. M. Y. Li for help in the preparation of this paper.

a series of intergrades between the monoecious and the gynoecious parents employed in each cross. Such sex intergrades are designated here as subgynoecists (Figure 4). The nature of the subgynoecious group has been a baffling problem in the genetic study of sex in cucumbers.

The source of andromonoecism (Figure 3*C*) is the old variety White Lemon and its derivative, the Australian variety Richmond Green Apple. Rosa[10] suggested that monoecism and andromonoecism are controlled by a pair of genes, monoecism being dominant. However, he classified only 16 F_2 plants.

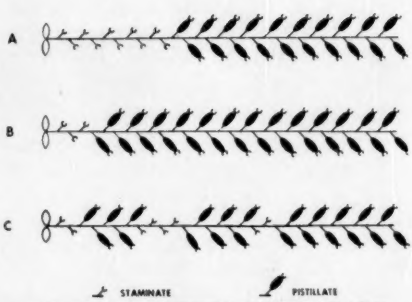
Hermaphrodites (Figure 3*D*) were developed recently[12] from crosses between gynoecists and andromonoecists. In these crosses one finds a graded series of andromonoecists which are designated here as subhermaphrodites. The latter are similar developmentally to subgynoecists.

Sex tendency is greatly affected by environmental factors. Among these are length of day, light intensity, and temperature[7, 16]. Long days, high light intensity, and high temperatures are conducive to strong male tendency, and the opposite conditions to weak male tendency. Furthermore, gibberellins[18, 19] increase and naphthalene acetic acid[5] decreases male tendency.

Experimental Approach and Material

In the present study an attempt was made to obtain more critical information about the relationship between: (a) the genes for different kinds of flowers, (b) the genes for sex tendency, (c) the gene associated with gynoecism, and (d) the environment.

The first step was to select monoecious inbreds which represent the wide



SUBGYNOECISTS OR INTERGRADE MONOECISTS

Figure 4

Intergrade phenotypes of patterns *A*, *B*, and *C* are often found in progenies of crosses between gynoecists and strongly male monoecists.

range of sex tendency among the cultivated varieties. The selected inbreds were MO-13, MO-7, and MO-96, having relatively weak, moderate, and strong male tendency, respectively. Inbred MO-13 was self-reproduced for 21 generations, MO-7 for 15 generations and MO-96 for 18 generations. In selecting these representative inbreds, the criteria for sex tendency were the average leaf numbers from the cotyledons to positions T1 and T2 on the main stem (Figure 3*A*). The T1 and T2 positions give better estimates of sex tendency than the T1 position alone.

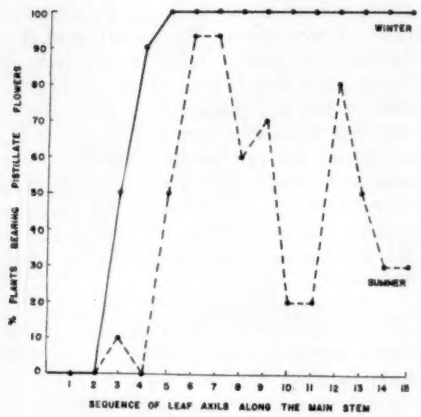
The T1 and T2 of the three inbreds are given in Table I for two seasons in New Brunswick, winter (greenhouse planting in late October with no supplementary light) and summer (field planting in early June).

The next step was to incorporate the gene associated with gynoecism into the genetic backgrounds of the three inbreds. This was accomplished and the new inbreds are described as follows:

GY-13 is a gynoecious inbred. It was

TABLE I. Sex tendency of three monoecious inbreds as measured by the average leaf number to the turning points (Figure 3*A*). Data based on 10 plants per inbred

Turning points	Winter			Summer		
	MO-13	MO-7	MO-96	MO-13	MO-7	MO-96
T1	6.9±1.4	8.8±1.8	17.7±3.5	18.3±3.0	21.9±2.9	34.1±3.0
T2	8.6±2.8	14.8±2.0	over 80	19.9±4.1	46.8±6.1	over 80



DEVELOPMENTAL BEHAVIOR

Figure 5

The graph illustrates developmental patterns of sex differentiation in inbred SGY-833 in two environments. See data in Table III.

developed from BC_8, gynoecist (gynoecist × MO-13) × MO-13, followed by five generations of inbreeding. Under summer conditions, the incidence of staminate flowers is estimated to be about 1:5000. It is extremely difficult to self-reproduce this inbred even with the aid of GA treatment[8, 15]. In this paper, GA treatment refers to the spraying of plants with an aqueous solution of 1.5 g/l of Merck's Gibrel, a potassium salt of gibberellic acid.

GY-7 is a gynoecious inbred. It was developed from BC_{11}, gynoecist (gynoecist × MO-7) × MO-7, followed by five generations of inbreeding. Under summer conditions, the incidence of staminate flowers is about 1:1000. This inbred can be self-reproduced readily with the aid of GA treatment. However, repeated spraying with GA leads to abnormal internode elongation, leaf distortion, and male sterility (staminate flowers devoid of androecium).

SGY-96 is a subgynoecious inbred of high phenotypic plasticity. It was developed from the cross GY-13 × MO-96, followed by five backcrosses, using MO-96 as a recurrent parent, and four generations of inbreeding. When MO-96 is used in backcrossing, the percent of gynoecists drops rapidly to zero and the percent of subgynoecists (Figure 4*A*) rises to 50 percent. From BC_3 on, one is compelled to use subgynoecists for backcrossing with MO-96.

The seed of Japanese and Korean gynoecious races was obtained from plant breeders[4, 6] and Japanese seed firms. The source of gynoecism used in the present study was a single female plant found in one of the Japanese varieties.

The andromonoecious inbred, ANDRO-23, of the variety Richmond Green Apple was used as parental material for perfect flowers. This inbred

TABLE II. Expression and inheritance of the factor associated with gynoecism. Summer, field culture. Phenotypic symbols: GY—gynoecious; SGY—subgynoecious; MO—monoecious

Tests in different genetic backgrounds	Offspring				
	GY	SGY	MO	Expected ratio (GY+SGY)/MO	P
Weak male tendency					
GY-13 × MO-13 (F_1)	117	2	0	---	---
F_1 × MO-13	1310	16	1291	1:1	.50
F_2 (summer, greenhouse)	139	12	55	3:1	.59
Moderate male tendency					
GY-7 × MO-7 (F_1)	52	39	0	---	---
F_1 × MO-7	247	181	415	1:1	.63
F_2	189	84	102	3:1	.35
Strong male tendency					
SGY-96 × MO-96 (F_1)	0	138	0	---	---
F_1 × MO-96	0	552	540	1:1	.73

is moderate to strong in male tendency. It was self-reproduced for three generations. The hermaphroditic inbred, H-25, was isolated as an F_6 selection from the cross GY-7 × ANDRO-23.

Results

Monoecism and gynoecism

A gynoecist of GY-13 was crossed with the three monoecious inbreds. The F_1 hybrids GY-13 × MO-13 (305 plants), GY-13 × MO-7 (287 plants), and GY-13 × MO-96 (362 plants) consisted of 98.0, 67.2, and 34.0 percent gynoecists, respectively. The remaining plants in each F_1 were subgynoecists of patterns *B* and *C* of Figure 4. These data were taken from field planting in summer.

In Table II, data are presented on the expression and inheritance of the factor associated with gynoecism, based on summer planting. The seed of the standard monoecious parent as well as remnant seed of the F_1 was planted along with the BC_1 and the F_2 generations of each cross. The presence of the monoecious parent and the F_1 hybrid in the same test helped in the identification of the sex phenotypes, particularly the subgynoecious grades. In general, subgynoecists can be distinguished from standard monoecists of the same genetic background by their lower T2 position and/or higher frequency of pistillate flowers along the main stem and branches.

The subgynoecists of the crosses GY-13 × MO-13 and SGY-96 × MO-96 were easily identified in this test. The subgynoecists of the cross GY-13 × MO-13 are very close phenotypically to gynoecists. They differentiate staminate buds at one to four basal leaf axils (Figure 4*B*) and some of these buds usually fail to reach anthesis. Furthermore, all branches of these subgynoecists are strictly female. The subgynoecists of SGY-96 × MO-96 reach the terminal female phase (Figure 4*A*) during

TABLE III. Developmental patterns of sex differentiation in inbred SGY-833 grown in two environments. Symbols: *=no visible flower buds or underdeveloped staminate buds; —=normal staminate flowers; +=normal pistillate flowers

Plant	Sequence of leaf axils along the main stem														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
							Winter								
1	*	*	+	+	+	+	+	+	+	+	+	+	+	+	+
2	*	*	+	+	+	+	+	+	+	+	+	+	+	+	+
3	*	*	*	+	+	+	+	+	+	+	+	+	+	+	+
4	*	—	+	+	+	+	+	+	+	+	+	+	+	+	+
5	*	*	*	+	+	+	+	+	+	+	+	+	+	+	+
6	*	*	*	—	+	+	+	+	+	+	+	+	+	+	+
7	*	*	+	+	+	+	+	+	+	+	+	+	+	+	+
8	*	*	+	+	+	+	+	+	+	+	+	+	+	+	+
9	*	*	—	+	+	+	+	+	+	+	+	+	+	+	+
10	*	*	*	+	+	+	+	+	+	+	+	+	+	+	+
% +	0	0	50	90	100	100	100	100	100	100	100	100	100	100	100
							Summer								
1	*	*	—	—	+	+	+	+	+	—	—	+	+	—	—
2	*	*	—	—	—	+	+	—	+	—	—	+	—	—	—
3	*	*	+	—	—	+	+	+	+	+	—	—	+	+	—
4	*	—	—	—	+	+	+	—	—	—	+	+	—	—	—
5	*	*	—	—	+	+	—	+	+	—	—	+	—	—	—
6	*	—	—	—	—	+	+	+	+	—	—	+	+	+	+
7	*	*	—	—	—	+	+	+	+	—	—	+	+	+	+
8	*	*	—	—	—	+	+	—	—	—	+	—	—	—	+
9	*	—	—	—	+	—	+	—	—	+	—	+	—	—	—
10	*	—	—	—	+	+	+	—	+	—	—	+	+	—	—
% +	0	0	10	0	50	90	90	60	70	20	20	80	50	30	30

the summer season, and therefore they are clearly distinguished from MO-96 (Table I).

In contrast to the above two crosses, it was difficult to classify the BC_1 and F_2 generations of the cross GY-7 × MO-7. The subgynoecists of this cross form a broad spectrum of intergrades (Figure 4 *A-C*), some of which are very close phenotypically to monoecists of MO-7. Nevertheless, it was possible to identify the subgynoecists during the summer season by following scrupulously the developmental patterns of individual plants and comparing these with those of MO-7.

Fifty plants of each of the three BC_1 generations were grown also during the winter season. The BC_1 of GY-13 × MO-13 consisted of 23 gynoecists, one subgynoecist, and 26 monoecists. The BC_1 of SGY-96 × MO-96 consisted of 22 subgynoecists and 28 monoecists. In both cases the classification of sex phenotypes did not present any difficulty. However, the BC_1 of GY-7 × MO-7 consisted of a continuous series of sex intergrades which the writer was unable to classify into qualitative groups even though MO-7 was present as a check.

It should be noted (Table II) that in cross GY-13 × MO-13 the percentage of subgynoecists among the heterozygotes of BC_1 grown in the field is about 1.2 percent (16 of 1,326 plants). In contrast, the estimated percentage of subgynoecists among the heterozygotes of the F_2 grown in the greenhouse is about 11.6 percent (12 of the expected 103 plants). The relatively high percentage of subgynoecists under greenhouse conditions during the summer months may be due to high daily temperatures, which often exceed 95° F.

New gynoecious and subgynoecious inbreds

Six new gynoecious and subgynoecious lines have been isolated from the cross GY-13 × MO-96, through inbreeding and selection. Of these, two are of special interest.

One inbred, GY-71, a gynoecious selection, produces numerous staminate flowers when treated with GA but, unlike other gynoecious lines, it does not exhibit abnormal internode elongation and leaf distortion following repeated GA spraying.

The other inbred, SGY-833, belongs to a unique group of subgynoecious lines. This inbred is strongly female in winter but in summer it manifests a pulsating pattern of sex differentiation (Table III, Figure 5). Other subgynoecious lines also exhibit the pulsating pattern in summer, but they are not as strongly female in winter. These subgynoecious inbreds differ from each other in the duration of the pulsating period. They eventually reach the strictly female phase (Figure 4*C*).

Although the environment greatly affects sex tendency, the orderly changes in sex differentiation during plant development may not be provoked necessarily by external variations. In this connection the following observation is significant. The monoecious inbred MO-7 was grown under controlled conditions of continuous illumination, 700 f.c. of fluorescent light at 70 to 75° F. Under these conditions, MO-7 exhibited a gradient course of differentiation which closely resembled its sex pattern in summer[15].

TABLE IV. Sex inheritance. Summer, field culture. Phenotypic symbols: GY—gynoecious; SGY—subgynoecious; H—hermaphroditic; SH—subhermaphroditic; MO—monoecious; ANDRO—andromonoecious

	Offspring						
Test	GY and SGY	H and SH	MO	ANDRO	Unclassified	Expected ratio	P
MO-13 × H-25 (F_1)	232	0	0	0	0	---	---
F_1 × MO-13	135	0	122	0	0	1:1	.44
F_1 × H-25	446	399	0	0	12	1:1	.21
F_1 × ANDRO-23	35	30	45	33	48	1:1:1:1	.32

It is evident that the expression of the factor associated with gynoecism varies in different genetic backgrounds. Monoecists which carry genes for a weak male tendency can be transformed into phenotypically stable gynoecists in the presence of a single dose of this factor. Monoecists which carry genes for a moderate male tendency can be transformed completely only in the presence of two doses. However, monoecists carrying genes for a strong male tendency may not be transformed into phenotypically stable gynoecists even in the presence of two doses. The data become more comprehensible if we assume that the genes for "sex tendency" (T1 and T2 in Figure 3) control the rate of sex conversion during development and that the factor associated with gynoecism accelerates this rate.

Hermaphroditism

The preceding interpretation of the role of the factor associated with gynoecism is further supported by the fact that hermaphrodites were found in the F_2 generations of crosses gynoecists $\times$ andromonoecists. However, it was not possible to obtain critical data on the inheritance of hermaphroditism in such crosses. The F_2 generations consisted of numerous sex intergrades and a relatively low percentage of hermaphrodites, averaging about three percent.

The low percentage of hermaphrodites in the F_2 generations could be attributed to the moderate and strong male backgrounds of the parents employed in these crosses. A very important modifying factor might be the strong male potency associated with the genetic factor for perfect flowers. In andromonoecious varieties, leaf axils (before T2) which differentiate perfect flowers usually differentiate staminate flowers also. In monoecious varieties, leaf axils which differentiate pistillate flowers seldom differentiate staminate flowers. Furthermore, it is difficult to establish hermaphroditic inbreds which are completely stable phenotypically under varying ecological conditions.

Inheritance of the four basic sex forms

The results of tests presented in Table IV prove that hermaphrodites carry the gene associated with gynoecism. In the tests presented in Table IV the four phenotypic groups were readily identified and the data indicate that these groups are governed by two independent genes. The unclassified plants recorded in Table IV were individuals which had not advanced sufficiently in development for accurate identification.

Thus, in a genetic background endowed with a relatively weak male tendency, the four basic sex forms behave in inheritance as fairly stable qualitative characters and these are associated with the following genes:

Gynoecism (and subgynoecism) ..... *Acr, G*
Hermaphroditism (and subhermaphroditism) ..... *Acr, g*
Monoecism (standard) ..... *acr, G*
Andromonoecism (standard) ..... *acr, g*

The symbol *Acr,* for an accelerator, is assigned to the gene which was previously associated only with gynoecism. This symbol is preferred to *D,* for dioecism[9]. The symbols *G* and *g* are assigned to the allelic genes governing the potentialities for pistillate and perfect flowers, respectively.

Subgynoecists and subhermaphrodites bridge the gap between gynoecists and standard monoecists and between hermaphrodites and standard andromonoecists. In a weak male background, they are very close phenotypically to gynoecists and hermaphrodites. However, they may be indistinguishable phenotypically from standard monoecists and andromonoecists of different genetic backgrounds. Therefore, subgynoecists and subhermaphrodites are defined as monoecists and andromonoecists which carry *Acr.* This gene separates them from standard *acr* races. A phenotypic definition of general applicability is difficult to find. Whether or not a monoecist (or andromonoecist) of unknown history carries *Acr* can be determined by

a breeding test, employing MO-13 (*acr/acr* in a weak male background) as a standard tester.

Discussion

The present hypothesis of sex control in cucumbers is based on available information and accepted genetic concepts. Accordingly, there are at least three groups of hereditary factors which govern sex expression during the development of a cucumber plant.

First, a few qualitative genes determine the kinds of flowers which can be differentiated potentially. All cucumber plants have the genetic potentiality to differentiate staminate flowers and, in addition, monoecists carry gene *G* for pistillate flowers and andromonoecists gene *g* for perfect flowers.

Second, polygenes govern the rate of accumulation or depletion of as yet unknown substrate. The concentration level of this substrate channels the expression of the genes for different kinds of flowers. The nature of the physiological process involved is not understood but some studies allude to the possible role of growth promoting and growth inhibiting substances in sex differentiation[2]. The basic assumption is that rate genes indirectly control "sex tendency."

Third, gene *Acr* markedly accelerates the rate of the physiological process controlled by the polygenes.

The entire pattern of sex differentiation during plant development depends upon the genes for different kinds of flowers, the polygenes, the dosage of *Acr*, and the environment.

Nongenetic factors such as photoperiodism, temperature, naphthalene acetic acid and gibberellins may affect the substrate which channels the action of the genes for different kinds of flowers.

The above hypothesis can explain the behavior of the existing sex variations in both *Cucumis sativus* L. and *Cucumis melo* L.[9, 12]. A similar hypothesis was offered to account for sex control in *Ricinus communis* L.[13]. The situation in *Ricinus* is of particular interest because the gene accelerator, or the group of accelerators, in this genus is brought about through the consequences of nuclear instability[11]. Whatever the genetic nature of these accelerators may be, there is little doubt that they will play a significant role in the breeding improvement of some cultivated species.

Summary

Sex in the cucumber plant, *Cucumis sativus* L., is a quantitative expression affected by genetic and nongenetic influences.

Standard monoecious and andromonoecious varieties differentiate staminate flowers early in development and later differentiate pistillate, *G*, or perfect, *g*, flowers in increasing number. The terminal phase in development is either strictly gynoecious or strictly hermaphroditic.

There exists a wide range of variation in male tendency among the cultivated races. The limited evidence suggests that several genes, perhaps polygenes, determine the rate of sex conversion during plant development and that this rate governs male tendency.

A rare major gene, *Acr*, markedly accelerates the rate of sex conversion. When this gene is combined with genes associated with different male tendencies, the outcome is a series of new sex variants, including gynoecious and hermaphroditic forms. Some of the new *Acr* inbreds exhibit pulsating patterns of sex differentiation.

The pattern of sex differentiation during plant development appears to depend upon an interaction between the genes for different kinds of flowers, the rate genes, the dosage of *Acr*, and the environment.

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A SELFING TUBE FOR MAIZE

G. N. STROMAN*

IN selfing maize plants, the usual method has been to bag the ear shoot with a four to six pound bag before the silks emerge, and to bag the tassel with a 10 to 12 pound bag at least 24 hours before the silks are ready for selfing. The pollen is then transferred to the ear shoot in a manner to thoroughly pollinate the silks and left until harvest time.

This method can be replaced by using a tube to connect the tassel bag and the ear shoot, so that as the silks emerge they will be pollinated immediately. As the wind blows, the pollen will sift down to the silks, and instead of small selfed ears one will obtain very large selfed ears. Also, another advantage is that when the tassel bag and the tube are in place, one need not visit the plant again until harvest time.

Making the Tubes

In order to make the tubes, a three-inch pipe about two meters long is mounted on a table so that about one and one-half meters protrude over the end of the table. Two or three bolts are sufficient to hold the pipe in place. Brown wrapping paper cut to size is placed around the protruding section of the pipe and secured by strips of gummed paper, thus forming a tube. In order to hold the tube open, a wire ring of bailing wire size is placed at one end and held in place by folding the paper back and ringing it with gummed paper. A wire ring can also be placed in the center of the tube, although this may not be necessary. These tubes can be made up very quickly and easily by laboratory helpers.

Putting the Tubes in Place

The tubes should be placed on the plants about the time one usually bags the ear shoot. The tassel bag is placed on the tassel. Then the end of the tube with the wire ring is placed in the tassel bag, and the tassel bag is drawn snug and stapled opposite the tube and also stapled to the tube. The other end of the tube is placed over the shoot and drawn down between the stem and shoot as one would ordinarily place the ear shoot bag. If the tube is too long, it is cut to size with a pair of scissors. It is then stapled securely but not too tightly.

SELFING MAIZE PLANTS

Figure 6

The tassel bag and tube are in place, and the plants need not be visited again until harvest time.

Figure 6 shows the tassel bag and tube in place. Such a tube was used successfully by the writer to inbreed some corn 20 years ago in New Mexico and recently at the Institute of Genetics in Peru. In this part of Peru it never rains, however, so whether the gummed paper will hold up in rainy climates is not known.

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HYBRIDS BETWEEN AGROPYRON REPENS AND AGROPYRON DESERTORUM

DOUGLAS R. DEWEY*

REPUTED natural hybrids between *Agropyron repens*, $2n=42$, and certain species of the "crested wheatgrass complex" including *A. cristatum*, $2n=14$, *A. pectiniforme*, $2n=28$, *A. sibiricum*, $2n=28$, and *A. desertorum*, $2n=28$, have been reported periodically. Peto[9] in an early survey of chromosome numbers of several *Agropyron* species observed two plants with 35 chromosomes from a seed lot of *A. repens* obtained from Georgia, U.S.S.R. On the basis of morphology and cytology he concluded that these plants must be natural hybrids of *A. repens* and a tetraploid crested wheatgrass, probably *A. sibiricum* or *A. desertorum*. He did not, however, confirm his observations with further investigation.

Troitsky[11] noted that, in areas in the U.S.S.R. where *A. cristatum* and *A. repens* grow together, plants that were transitional between the two species could be found. Some of the intermediate types could not be distinguished from *A. sibiricum*. This last species was thus considered to be a product of natural interspecific hybridization. This same view was also expressed by Peto[9] and Tronichij[12]. Kotov[5] found in the Ukraine a series of plants that resembled *A. cristatum*, *A. sibiricum*, and *A. repens* which he considered to be hybrids of *A. repens* × *A. pectiniforme*. Except for Peto's investigation, none of the reports of natural hybridization of *A. repens* with crested wheatgrass were supported by cytological data, and in no case were controlled crosses of these species made in an attempt to duplicate what reputedly had occurred naturally.

In 1952, Charles Marshall, Chief, Regional Nursery Division, S.C.S., Albuquerque, New Mexico, reported some putative hybrids of *A. cristatum* × *A. trichophorum*, *A. intermedium* or *A. repens*. His report was unique in that he had the specific *A. cristatum* plant that entered into this apparent hybridization. When the S.C.S. Albuquerque nursery was abandoned in 1954, Mr. Marshall sent clonal pieces of the parent plant, *A. cristatum*, and 15 putative hybrids to research personnel at the Utah Agricultural Experiment Station, Logan, Utah, so that the material would not be lost. The receipt of Mr. Marshall's plant material stimulated interest in the validity of this and other reports of natural interspecific hybridizations involving a species of crested wheatgrass and a rhizomatous *Agropyron*, most likely *A. repens*. This paper reports the results of an investigation designed to duplicate under controlled conditions results of reported natural hybridizations between *A. repens* and tetraploid crested wheatgrass and at the same time explore the genome constitution of these two species.

Materials and Methods

The confused taxonomic status of the crested wheatgrasses makes the exact identification of the species referred to in the literature questionable even with respect to chromosome number. In this paper the crested wheatgrasses are considered to consist of two basic species—*A. cristatum*, $2n=14$, and *A. desertorum*, $2n=28$. Such a classification appears to be realistic inasmuch as the tetraploid species have been shown by Knowles[4] to be generally cross-fertile and produce fertile progeny with a meiosis typical of normal tetraploid crested wheatgrass. Crosses of the diploid species with the tetraploids were not usually successful, and the few progeny obtained were sterile.

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In the grass nurseries at the Evans Farm of the Utah Agricultural Experiment Station, *A. repens* and *A. desertorum* grow in close proximity. The species flower simultaneously and provide an ample opportunity for natural interspecific hybridization. Open-pollinated seed was collected from 13 plants of *A. repens* to determine whether natural hybrids were being produced in our nurseries. This seed was planted in sterilized greenhouse flats in February 1957, and the following spring 755 seedlings from this seed were transplanted to the field for observation and classification as hybrid or non-hybrid.

During the winter of 1957-1958 a large number of controlled crosses were made in the greenhouse among several plants of *A. repens* and *A. desertorum*. The female parent, *A. repens*, was hand-emasculated several days before anthesis, after which the spike was covered with a glassine bag to exclude foreign pollen. Pollination was achieved by inserting a detached, pollen-bearing spike of *A. desertorum* into the bag with the emasculated spike of *A. repens*. The detached spike was placed in a vial of water so that it would continue to shed pollen for several days.

For cytological studies, spikes were fixed in Newcomer's solution[7] and stored under refrigeration for five to six months before observations were made. The aceto-carmine stain technique was used in all chromosome studies.

All plant material used in this study is currently maintained in field nurseries and voucher specimens will be placed on file with the Intermountain Herbarium, Logan, Utah.

Results

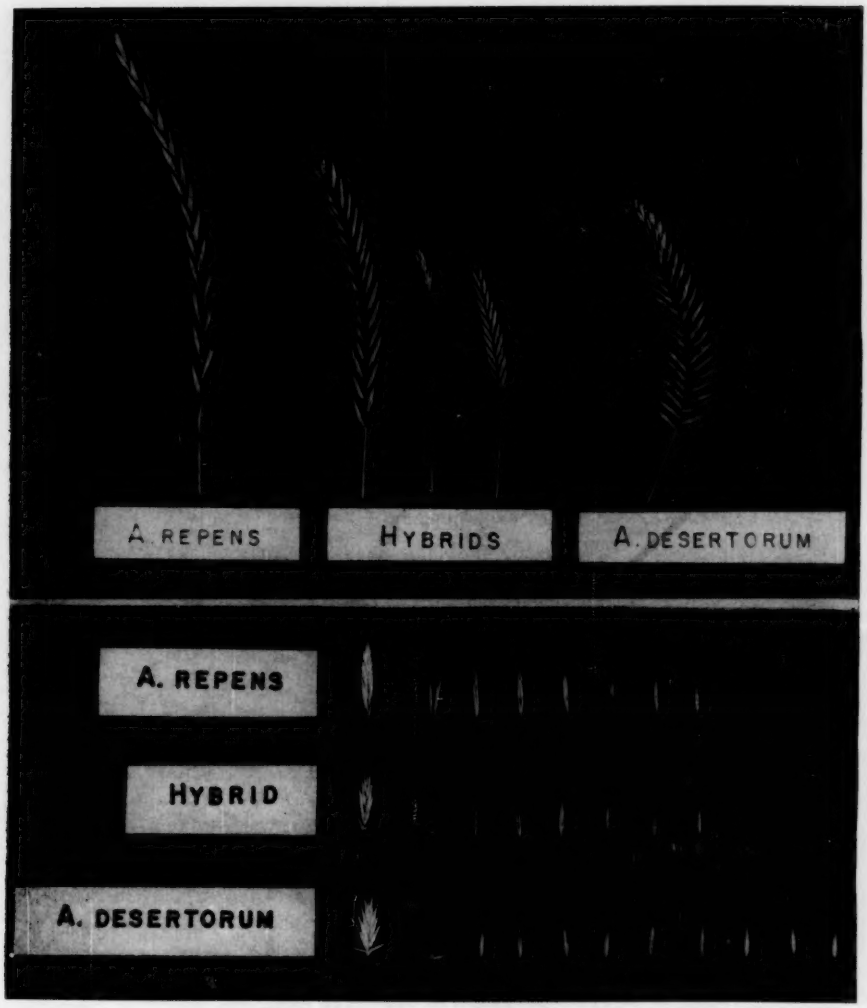
Marshall's putative hybrids

Cytological analysis of the clonal material obtained from Charles Marshall revealed that the parent plant identified as *A. cristatum* was actually a tetraploid, $2n=28$, and would be more accurately identified as *A. desertorum*. Of the 15 plants designated as hybrid progeny of the parent *A. desertorum* plant, only two appeared morphologically to be hybrids. When examined cytologically, these plants were found to have 35 chromosomes, exactly what one would expect from a hybridization of *A. desertorum* × *A. repens*. Meiotic irregularities—univalents, laggards, and micronuclei — further indicated that these two plants were in reality hybrids. The remaining 13 plants contained 42 chromosomes, and their meiotic behavior was typical of *A. repens*. These plants produced vigorous rhizomes, bore all the morphological characteristics of *A. repens* and have been identified as *A. repens* by A. H. Holmgren, curator of the Intermountain Herbarium, Logan, Utah. It has now been confirmed that a natural hybridization had occurred between *A. desertorum* and a rhizomatous *Agropyron*, likely *A. repens*.

Natural and controlled hybrids of A. repens × A. desertorum

Of 755 open-pollination progeny of 13 *A. repens* plants grown adjacent to a planting of *A. desertorum*, 28 bore a strong resemblance to *A. desertorum*, particularly with respect to spike characters. These plants possessed small rhizomes, were morphologically intermediate between *A. repens* and *A. desertorum* and were undoubtedly hybrids. The hybrid plants were essentially sterile but not completely so. In this instance the *A. repens* parent plants were known with certainty; and although the evidence pointing to *A. desertorum* as the other parent species is entirely circumstantial, there is virtually no doubt that *A. desertorum* was the male parent involved in the cross. Natural crossing occurs quite readily between these two species inasmuch as 3.7 percent of the open-pollination progeny were hybrid. Some *A. repens* plants produced over 10 percent natural hybrids, while others produced none.

The most critical information comes from the controlled hybrids produced in the greenhouse where both parent plants were known with certainty. From 54 crosses of *A. repens* × *A. desertorum*, which involved 1,517 hand-emasculated florets, 219 seeds were obtained. Some crosses resulted in more than 50 per-



SPIKES OF PARENT AND HYBRID PLANTS

Figure 7

In the upper half of the figure appear typical spikes of *A. repens, A. desertorum,* and three of their interspecific hybrids, and in the lower half, individual spikelets of *A. repens, A. desertorum,* and the interspecific hybrid.

cent seed set, while others were completely unsuccessful. The average seed set was 14.4 percent. Of the 219 seeds obtained only 18 germinated. Poor germination is a characteristic of *A. repens,* and the poor germination of the hybrid seed was not entirely unexpected. If the cross was made with *A. desertorum* as the female parent, germination of hybrid seed might be increased.

Morphology of A. repens, A. desertorum and hybrids

The controlled hybrids could not be distinguished morphologically from the natural hybrids, and all tended to re-

semble *A. desertorum* more than *A. repens*. Typical spikes of the parent species and three hybrids and individual florets are pictured in Figure 7. Measurements of spike, spikelet, and floret characteristics of four controlled hybrids and their parents are summarized in Table I. Ten representative spikes of each plant were measured. The hybrids differed rather widely with respect to leaf and spike characteristics and size. For instance, hybrid 173 × 215B was a miniature plant with unusually poor vigor and was much smaller than either parent, while hybrid 12 × 172 was a large robust plant with vigor equal to or greater than that of either parent. This variation in general appearance and morphological characteristics illustrates the hazard of describing a hybrid on the basis of a single plant.

Hybrid 12 × 172 was intermediate between the parent plants for all characters measured except spikelet and lemma lengths. This was also true of hybrid 13 × 159 and hybrid 5 × 172. Hybrid 173 × 215B, the miniature plant, tended to be smaller in all respects than either parent. Absolute measurements of spike length, width, and other forage and spike characters are not nearly as important in describing the features of a plant as are ratios of these absolute measurements. Absolute values can be easily influenced by the conditions under which the plant is grown and by genetic factors which may cause dwarfing, while ratios tend to be much more stable. When the ratios in Table I are compared, it will be noted that the hybrids are invariably intermediate between the parent species. With a single exception, the hybrids resembled *A. desertorum* more closely than *A. repens* for all measurements given as ratios.

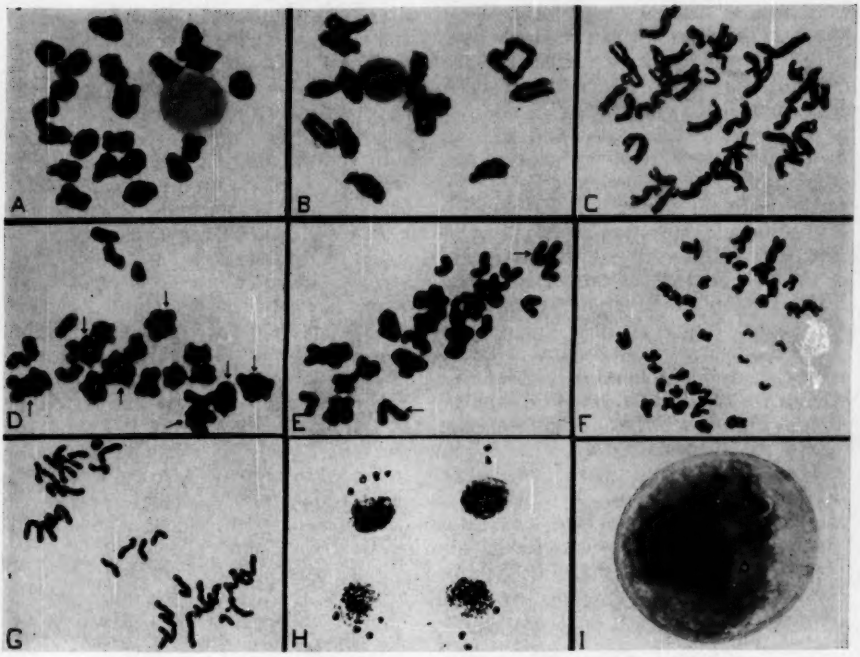
Cytology of A. repens, A. desertorum and hybrids

Results of a cytological analysis of the *A. repens* and *A. desertorum* parent plants are given in Table II. One hundred and four cells from three *A. repens* plants were completely interpreted at diakinesis or metaphase I to determine

TABLE I. Spike characteristics of *A. repens*, *A. desertorum*, and four of their interspecific hybrids

	Cross No. 1			Cross No. 2			Cross No. 3			Cross No. 4		
Character	*A. repens* (12)	*A. desertorum* (172)	Hybrid (12 × 172)	*A. repens* (13)	*A. desertorum* (159)	Hybrid (13 × 159)	*A. repens* (173)	*A. desertorum* (215B)	Hybrid (173 × 215B)	*A. repens* (5)	*A. desertorum* (172)	Hybrid (5 × 172)
Spike length, cm.	11.30	8.26	9.50	10.88	8.93	8.36†	16.54	9.80	5.05†	13.16	8.26	10.63
Spike width, cm.	.48	2.16	1.08	.42	1.63	.85	.74	2.38	.58†	.57	2.16	1.26
Spike length:width, ratio	23.54	3.82	8.80	25.90	5.48	9.84	22.35	4.11	8.71	23.08	3.82	8.44
Spikelets/spike, no.	18.0	49.5	20.0	17.3	44.8	29.6	28.0	43.8	25.0	†23.2	49.5	33.1
Spike internode length, cm.	.66	.17	.50	.67	.20	.29	.61	.22	.20†	.59	.17	.33
Spikelet length, cm.	1.29	1.29	1.65†	1.22	1.20	1.14†	1.83	1.60	.84†	1.41	1.29	1.53†
Spikelet width, cm.	.38	.87	.58	.36	.72	.50	.42	.82	.34	.30	.87	.50
Spikelet length:width, ratio	3.39	1.48	2.84	3.39	1.67	2.28	4.36	1.95	2.47	4.70	1.48	3.06
Florets/spikelet, no.	4.8	6.6	6.0	4.2	7.0	4.4	7.6	11.0	3.6 †	6.1	6.6	6.4
Glume length, cm.	.93	.66	.90	.97	.68	.70	1.08	.77	.57†	.85	.66	.80
Glume awn length, cm.	.11	.24	.22	.15	.17	.16	.11	.26	.11†	.09	.24	.17
Glume:glume awn, ratio	8.45	2.75	4.09	5.80	4.00	4.38	9.82	2.96	5.18	9.44	2.75	4.70
Glume angle*, degrees	19.0	134.1	34.0	20.7	135.7	53.6	23.3	67.2	39.6	16.8	134.1	35.9
Lemma length, cm.	1.04	.94	1.12†	1.03	.95	.86†	1.06	.86	.70†	.99	.94	1.07†
Lemma awn length, cm.	.04	.20	.19	.04	.16	.09	.03	.23	.12	.03	.20	.18
Lemma:lemma awn, ratio	26.00	4.70	5.89	25.75	5.94	9.56	35.33	3.73	5.83	33.00	4.70	5.94

*Glume angle refers to the angle formed by the junction of the two glumes with each other.
†Characters for which the hybrid is not intermediate between the parents.



CYTOLOGY OF *A. REPENS*, *A. DESERTORUM* AND HYBRIDS

Figure 8

A—Diakinesis of *A. repens* showing 21 bivalents. *B*—Diakinesis of *A. desertorum* with 10 bivalents and two quadrivalents. *C*—Mitotic metaphase of hybrid. Note the difference in size of chromosomes. Fourteen large chromosomes are from *A. desertorum* and 21 small chromosomes are from *A. repens*. *D*—Metaphase I of hybrid with 14 bivalents and seven univalents. Seven large bivalents indicated by arrows are *A. desertorum* chromosomes. *E*—Metaphase I of hybrid with 13 bivalents and nine univalents. Seven small univalents are *A. repens* chromosomes and the two large univalents indicated by arrows are from *A. desertorum*. *F*—Anaphase I of hybrid with seven lagging chromosomes. Two of the lagging dyads are dividing precociously. *G*—Anaphase II of hybrid with four lagging chromosomes. *H*—Quartet of hybrid with 13 micronuclei. *I*—Immature pollen grain of hybrid with five micronuclei.

chromosome associations. The most frequent chromosome association was 21 bivalents, although 35 percent of the cells contained one or more quadrivalents with one cell having a maximum of four quadrivalents. Figure 8*A* shows 21 bivalents of *A. repens* at diakinesis. Laggards appeared in approximately three percent of the cells at anaphase I and II, and less than one percent of the quartets had micronuclei. Other *A. repens* plants were found to show much greater meiotic irregularity, but these plants were avoided in these species crosses so as not to confound meiotic irregularities of the parent species with irregularities occurring in the hybrid as a result of hybridization.

Fifty-four cells of *A. desertorum* were interpreted at diakinesis and early metaphase I (Table II). Fifty-three of the 54 cells contained one or more quadrivalents, and the average number of quadrivalents per cell was 2.37. Figure 8*B* shows a cell of *A. desertorum* at diakinesis with 10 bivalents and two quadrivalents. The chromosomes of *A. desertorum* are about twice the size of those of *A. repens*. This size difference is extremely important in interpretation of chromosome homologies and the nature of pairing in the hybrid. No univalents

were seen at diakinesis or early metaphase I; however, at late metaphase I a few chromosomes appeared off the metaphase plate and may have been either univalents or chromosomes loosely associated at diakinesis and passing to the poles slightly in advance of the other dyads. In any event, cells of this nature were not included in the cytological studies. Meiosis was essentially regular with approximately three percent of the cells having laggards at anaphase I and II. A few micronuclei were observed in the quartets.

All the hybrids, both natural and controlled, had 35 chromosomes. A mitotic metaphase of one of the hybrids can be seen in Figure 8*C*. Note the difference in size of the various chromosomes. The smaller chromosomes were contributed by the *A. repens* parent. A summary of the meiotic behavior of six hybrids is given in Table III. The data for the six plants were combined since all hybrids behaved much the same in meiosis. Two hundred and thirty-five cells were interpreted completely at diakinesis and metaphase I to determine pairing relationships in the hybrids. The most common association, 14 bivalents and seven univalents, was observed in 56.6 percent of the cells. Seven bivalents were much larger than the other seven, and the univalents as a rule were small chromosomes. The large bivalents were apparently *A. desertorum* chromosomes that were pairing autosyndetically, while the seven small bivalents, also the result of autosyndesis, and the seven univalents were *A. repens* chromosomes. Metaphase I in the hybrid is illustrated in Figure 8*D*. Seven large bivalent chromosomes of *A. desertorum* are indicated by arrows. In no case were large chromosomes found pairing with small.

The next most frequent chromosome association was 13 bivalents and nine univalents, 22.6 percent of the total. Figure 8*E* shows this association. Seven of the univalents were of the small variety, *A. repens;* and two univalents, as indicated by arrows, were the large type, *A. desertorum.* Trivalents appeared in 13.2 percent of the cells and one quadrivalent was found in two cells.

Laggards appeared in every cell at anaphase I. Although the most common number of laggards per cell was seven, the average was 6.15. This was somewhat less than the number of univalents recorded at metaphase I. Apparently a few univalents passed normally to the poles at anaphase I. A typical anaphase I is shown in Figure 8*F*. This cell has 14

TABLE II. Meiotic chromosome behavior of *A. repens* and *A. desertorum*

	Chromosome Associations			Anaphase I		Anaphase II		Quartets	
	II	IV	No. of cells	Laggards/cell	No. of cells	Laggards/cell	No. of cells	Micronuclei quartet	No. of quartets
				Agropyron repens					
	21	0	68	0	263	0	137	0	334
	19	1	28	1	5	1	3	1	0
	17	2	6	2	5	2	1	2	2
	15	3	1						
	13	4	1						
Total	2090	47	104	15	273	5	141	4	336
Average	20.10	.45		.05		.04		.01	
				Agropyron desertorum					
	14	0	1	0	171	0	164	0	165
	12	1	13	1	4	1	6	1	1
	10	2	17	2	1			2	4
	8	3	14					3	0
	6	4	6					4	1
	4	5	33						
Total	500	128	54	6	176	6	170	11	171
Average	9.26	2.37		.03		.03		.06	

chromosomes passing to each pole and seven lagging dyads of the small type. Two of these dyads are dividing precociously. Precocious division of lagging dyads was the general rule, and in many cells all the laggards were dividing at the first anaphase. Lagging was also very common at anaphase II, with an average of 4.88 per cell. Four lagging chromosomes are visible in Figure 8*G*.

Micronuclei were very common in the quartets and averaged 7.34 per quartet. If each laggard at anaphase II formed a micronucleus, one would expect an average of 9.76 micronuclei per quartet. Either some of the laggards eventually were included in the major nucleus or some of the micronuclei consist of more than one chromosome. Figure 8*H* shows a quartet with 13 micronuclei. A count of over 4,000 pollen grains gave 17.3 percent normal-sized, stainable pollen. An immature pollen grain with five micronuclei can be seen in Figure 8*I*. Although the hybrid plants were highly sterile, 186 viable seeds were obtained from 24 hybrids.

Discussion

The meiotic behavior of *A. desertorum* reported herein is typical of that observed elsewhere. Knowles[4] reported an average of 9.1 bivalents, 2.2 quadrivalents, and occasional univalents and trivalents per cell as compared with 9.3 bivalents and 2.4 quadrivalents observed in the present study. Myers and Hill[6] noted a somewhat higher quadrivalent frequency of 3.7 per cell. Both papers suggested that tetraploid crested wheatgrasses are probably autopolyploids. Hybrids of *A. cristatum* ($2n=14$) × *A. desertorum* ($2n=28$) were found by Knowles[4] to contain a high frequency of trivalents, which further indicates that *A. desertorum* is an autotetraploid. Further proof of the autoploid nature of *A. desertorum* comes from a polyhaploid plant of this species located by the author, in which meiotic pairing was very good with 62 percent of the cells having seven bivalents. Designating *A. desertorum* with the genomic formula AAAA seems fully justified when results of these various investigations are examined.

The type of polyploidy in *A. repens* is not as well documented as that in *A. desertorum*. The occurrence of occasional quadrivalents in this species indicates either autoploidy or heterozygous reciprocal translocations. Since within pollen-mother-cells of a given plant, quadrivalent frequency ranged from none to four, autoploidy rather than heterozygous reciprocal translocations would seem to be involved. Ostergren[8] observed a few quadrivalents in *A. repens*, but he attributed them to structural hybridity. Godley[3] assigned a genomic formula of $A_1A_1A_{2s}A_{2s}CC$ to *A. repens*. He considered

TABLE III. Meiotic chromosome behavior of hybrids of *A. repens* × *A. desertorum*

Chromosome Associations					Anaphase I		Anaphase II		Quartets	
I	II	III	IV	No. of cells	Laggards/ cell	No. of cells	Laggards/ cell	No. of cells	Micro-nuclei/ quartet	No. of quartets
7	14			133	2	1	0	7	0	13
9	13			53	3	7	1	15	1	23
6	13	1		17	4	13	2	33	2	34
5	15			12	5	34	3	61	3	36
8	12	1		12	6	56	4	71	4	36
11	12			3	7	60	5	53	5	38
13	11			2	8	25	6	71	6	33
5	13		1	1	9	16	7	68	7	36
8	10	1	1	1	10	8	8	20	8	35
10	11	1		1	14	1	9	16	9	41
							10	2	10	29
									11	35
									12	25
									13	26
									14	13
									15	10
									16	5
									17	1
Total 1748	3188	31	2	235	1439	234	2039	417	3509	478
Average 7.44	13.57	.13	.009		6.15		4.88		7.34	

the two A genomes as having arisen from two ecospecies and thus basically homologous. The third genome, designated as the C genome, was apparently contributed by a distinctly different diploid species. Cauderon[1] gave *A. repens* the genome formula of $R_1R_1R_2R_2Z_1Z_1$, which in principle is the same as Godley's. The two R genomes were thought to have arisen from a single diploid species, although they are not completely homologous.

In most cytological investigations of interspecific and intergeneric hybrids, the inability to distinguish between chromosomes of two species in their hybrids has placed a severe limitation on the conclusions drawn with respect to genome homologies of the different species involved. Under such circumstances, one can only speculate whether pairing indicates genome homologies of the different species or is the result of autosyndesis among chromosomes of the same species. In most studies, pairing in such hybrids has been interpreted to be the result of chromosome homologies between the species or the genera involved in the hybridization. Because of the size difference of *A. repens* and *A. desertorum* chromosomes, the present study provides critical proof as to the type of chromosome pairing that occurs in the hybrid. Peto[9] recorded similar size differences between chromosomes of these same species, but he did not make use of it in interpreting his results.

Observations at diakinesis and metaphase I of the *A. repens* × *A. desertorum* hybrids clearly revealed that chromosome pairing was exclusively autosyndetic. The most frequent chromosome association of 14 bivalents and seven univalents consisted of seven bivalents resulting from autosyndesis of *A. desertorum* chromosomes, seven bivalents of *A. repens* also formed by autosyndesis and seven *A. repens* univalents. The seven univalent *A. repens* chromosomes represent a genome distinctly different from any other found in these two species. One must conclude that *A. repens* and *A. desertorum* do not contain any genomes in common. In cells in which less than 14 bivalents were formed, the usual pattern was seven *A. desertorum* bivalents and fewer *A. repens* bivalents. Apparently two genomes of *A. repens* are very closely related but are not completely homologous. This would account for the relative rarity of quadrivalent formation in *A. repens* itself in which preferential pairing results in almost all bivalents. Stebbins and Pun[10] reported a situation in *A. intermedium* similar to that found in *A. repens*. *A. intermedium*, like *A. repens*, normally forms 21 bivalents at diakinesis, but in an intergeneric hybrid with *Secale cereale*, 14 of the *A. intermedium* chromosomes paired autosyndetically to form seven bivalents. They concluded that *A. intermedium* consisted of two genomes which were strongly though not completely homologous, plus a third genome which had only a slight degree of homology with the other two. This is the same condition that exists in *A. repens*.

On the basis of the results obtained in the present study, the interspecific hybrid of *A. repens* × *A. desertorum* can be assigned the genome formula of AAB_1B_2C. The A genomes were contributed by *A. desertorum*, AAAA, and the B and C genomes were derived from *A. repens*, $B_1B_1B_2B_2CC$. These genome formulae are in complete harmony with related work of Myers and Hill[6], Godley[3], Cauderon[1], and Knowles[4].

These results cast considerable doubt upon conclusions reached in some other investigations. For instance, Favorsky[2] reported a cross of *Secale cereale*, $2n=14$, and *A. cristatum*, $2n=28$, and found that seven bivalents and seven univalents were regularly formed in the hybrid. He assumed that the chromosomes of *S. cereale* were homologous with seven of the 14 chromosomes from the tetraploid crested wheatgrass. A more accurate interpretation would be that the seven bivalents were the result of autosyndesis of crested wheatgrass chromosomes, and the *S. cereale* chromosomes were left as unpaired univalents. This would lead one to conclude that no homologies exist between *S. cereale* chromosomes and those of tetraploid crested wheatgrass. Stebbins and Pun[10] suggested the same interpretation of Favorsky's results.

It can be safely said that in interspecific or intergeneric hybrids involving either *A. repens* or *A. desertorum* part if not all of the chromosome pairing will be the result of autosyndesis. This fact should aid in providing a more accurate interpretation of results of past investigations and act as a guide for future studies.

Summary

A report by Charles Marshall, Chief, Regional Nursery Division, S.C.S., Albuquerque, New Mexico, of 15 putative hybrids of *A. desertorum* and a rhizomatous *Agropyron*, probably *A. repens* or *A. trichophorum*, was found to be partially accurate. A cytological analysis revealed that two of the reputed hybrids contained 35 chromosomes and were actually hybrids, while the remaining 13 plants were identified simply as *A. repens* on the basis of cytological and morphological data.

Natural hybridization occurred readily between *A. repens*, $2n=42$, and *A. desertorum*, $2n=28$, when these two species were grown in close proximity. Nearly four percent of the open-pollination progeny from *A. repens* was found to be natural hybrids.

Controlled crosses between *A. repens* and *A. desertorum* were successful. Seed set in these crosses was 14.4 percent, but germination was poor. Most hybrids, natural and controlled, were intermediate between the parent species with respect to morphological charac-

teristics of the spike. The hybrids were rhizomatous but not nearly so aggressive as *A. repens*.

A. repens contained 42 chromosomes, *A. desertorum* had 28, and all hybrids had 35 chromosomes. A size difference between chromosomes of *A. repens* and *A. desertorum* permitted these chromosomes to be distinguished in the hybrid. The most frequent chromosome association at diakinesis and metaphase I was 14 bivalents and seven univalents, which comprised 56.6 percent of the 235 cells interpreted. Chromosome pairing in the hybrid was determined to be completely autosyndetic, with the 14 *A. desertorum* chromosomes usually forming seven bivalents and 14 of the 21 *A. repens* chromosomes doing the same and leaving seven *A. repens* chromosomes as univalents.

On the basis of these cytological investigations, *A. desertorum* was given the genome formula of AAAA, while *A. repens* was designated $B_1B_1B_2B_2CC$ and their hybrids AAB_1B_2C. The application of these findings to other investigations was discussed.

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(*Continued on page* 38)

ORIGIN OF GENETIC CONTROL OF DIPLOID-LIKE BEHAVIOR OF POLYPLOID WHEAT

RALPH RILEY, GORDON KIMBER AND VICTOR CHAPMAN*

WORK with 20-chromosome nulli-V haploids and 40-chromosome V-nullisomics has demonstrated that the purely bivalent-forming meiotic organization of common wheat, *Triticum aestivum* ($2n = 6x = 42$), is controlled by one or more genes on chromosome 5[1,2]. Simultaneous confirmation of this was provided by work on hybrids deficient for chromosome 5[7], and subsequent investigation has shown that this chromosome alone is concerned in the diploidization of wheat[2]. When chromosome 5 is absent, corresponding homoeologous chromosomes from the three different genomes can pair at meiosis; in its presence this does not normally occur. The effective gene, or genes, on chromosome 5 thus provide the potentiality of high fertility and the genetic stability which have made *T. aestivum* a successful polyploid and have allowed it to develop into one of the major crop plants of the world.

It is immediately pertinent to ask, therefore, whether the control exercised by chromosome 5 was developed subsequent to the origin of the polyploid state, or whether it occurred as a fortuitous result of the incorporation of an unaltered chromosome from a diploid ancestor. Chromosome 5 is in the B genome[6] and so was brought with that genome into the tetraploid wheat species, and from the tetraploids into the hexaploids. The B genome was probably provided by a diploid species in the Sitopsis section of the genus *Aegilops*[4,5]. It is therefore relevant to examine the Sitopsis section for indications of genetic effects on chromosome pairing similar to those caused by chromosome 5.

The Sitopsis section contains only diploid species. They are *A. speltoides, A. bicornis, A. longissima* and *A. sharonensis,* although the last two species are extremely closely related, forming fertile hybrids, and probably constitute a single genetic species. As pointed out by Riley *et al.*[3], there are important differences between the Sitopsis species in the influence they have, in hybrids with tetraploid wheat, on the pairing of wheat chromosomes. Triploid hybrids, involving *A. longissima, A. bicornis* or *A. sharonensis* and tetraploid wheat, have very little meiotic chromosome pairing. The *Aegilops* chromosomes rarely pair with the wheat chromosomes and the wheat chromosomes rarely pair with each other.

By contrast, when *A. speltoides* is crossed with a tetraploid wheat there are completely different pairing relationships in the triploid hybrid. Not only do the *A. speltoides* chromosomes pair with wheat chromosomes but wheat chromosomes pair with each other. Trivalents are common, and these must arise from the conjugation of structurally corresponding chromosomes in the two wheat genomes together with the related *Aegilops* chromosome.

The *A. speltoides* genotype thus breaks down the meiotic isolation of homoeologous chromosomes in tetraploid wheat, just as the removal of chromosome 5 does in hexaploid wheat. However, the chromosome 5 condition of the hexaploids is also repeated in tetraploid wheat since no segregants with homoeologous pairing are produced in tetraploid-hexaploid crosses. Thus, some component of the *A. speltoides* genotype suppresses the effect of the chromosome 5 gene. It appears, therefore, that the

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other Sitopsis species, which do not alter the normal meiotic affinities, are similar to the present condition of wheat in the genetic control of chromosome pairing. Consequently, *A. speltoides* seems less likely to be the B genome donor, despite the favorable evidence of karyotype and external plant morphology[3, 4].

To clarify this situation the genetic relationships have been investigated between the pairing control exercised by chromosome 5 of hexaploid wheat and the influence of two different species in the Sitopsis section of *Aegilops*. The aim of this work was to study meiotic pairing in hybrids, between wheat and the *Aegilops* species, with and without chromosome 5 of wheat.

Materials

To obtain hybrids deficient for chromosome 5, crosses were made using plants of *T. aestivum* monosomic for chromosome 5 as female parents. These were pollinated by the *Aegilops* parent, so that the same cross could give rise to hybrids with and without chromosome 5.

The monosomic 5 parents were in *Triticum aestivum* L. variety Chinese Spring, and were kindly supplied by Dr. E. R. Sears. However, in the case of crosses between monosomic 5 and *A. speltoides*, only 27-chromosome hybrids, deficient for 5, were produced. Consequently, the 28-chromosome class was completed using hybrids of which the female wheat parent was *T. aestivum* L. variety Holdfast ($2n = 42$). The change of variety is not likely to have affected pairing significantly since Chinese Spring and Holdfast are essentially similar in chromosome structure, differing only in a reciprocal translocation involving chromosomes 3 and 17.

The two *Aegilops* species of the Sitopsis section involved in hybrids with *T. aestivum* were *A. longissima* Schweinf. and Muschl. ($2n = 14$) and *Aegilops speltoides* Tausch *ligustica* Savign. ($2n = 14$).

Meiosis was studied in Feulgen-orcein stained squashes of pollen-mother-cells from anthers fixed in acetic alcohol.

Results

First of all it should be indicated (Table I) that the two Sitopsis species *A. speltoides* and *A. longissima* were very similar in chromosome structure. Pairing was very good in hybrids between them, apart from the interference caused by heterozygosity for a reciprocal translocation. Thus, the differences in pairing between their hybrids and polyploid wheat species are not related to structural divergence.

The genetic differences between *A. speltoides* and *A. longissima,* which show in hybrids with tetraploid wheats, were also apparent in 28-chromosome hybrids with *T. aestivum* (Table II). The 28-chromosome hybrids with *A. speltoides* had very high pairing with many trivalents and quadrivalents. In a few cells every chromosome was conjugated; for example, one had five bivalents, two trivalents and three quadrivalents. Homoeologous chromosomes from all three wheat genomes, as well as the matching *A. speltoides* chromosome, must have participated in the quadrivalents. By contrast the 28-chromosome hybrid with *A. longissima* had very little pairing—hardly more than might be expected in a euhaploid of wheat. There were never more than 12 chromosomes paired in any cell. Clearly the *A. speltoides* genotype removes the inhibition of homoeologous pairing caused by chromosome 5 of wheat, but the *A. longissima* genotype does not.

However, there were no major differences between the 27- and 28-chromosome hybrids with *A. speltoides*. Re-

TABLE I. Mean chromosome pairing at metaphase I of meiosis in an *A. longissima* × *A. speltoides* F_1 hybrid

		Bivalents				
Cells	univ.	rod	ring	total	triv.	quad.
50	0.54	2.32	2.86	5.18	0.34	0.52

moval of wheat chromosome 5 produced no modification of pairing, presumably because its influence was already suppressed by the *A. speltoides* genotype. By contrast the 27-chromosome hybrids with *A. longissima* had much higher pairing than the normal 28-chromosome hybrids. There were numerous trivalents and quadrivalents and there were occasional cells without any univalents. Apparently, as in nulli-V haploids, homoeologous pairing could then take place.

Discussion

The effect of the *A. longissima* genotype is like that of wheat chromosome 5 in that neither breaks down the meiotic isolation of homoeologous chromosomes. There is a distinction, nevertheless, in that chromosome 5 produces a positive effect — homoeologous pairing only occurring in its absence. When chromosome 5 is absent, however, the *A. longissima* genome cannot prevent homoeologous pairing in hybrids. Thus, there is nothing in the *A. longissima* genotype which can compensate for the absence of chromosome 5. Consequently, whatever the fundamental effect of chromosome 5 may be, it is not produced by *A. longissima*. The *A. speltoides* and *A. longissima* genotypes, and chromosome 5 of wheat, all operate differently in their influence on chromosome pairing.

A. bicornis is like *A. longissima* in lacking the capacity to compensate for the absence of chromosome 5 (Riley unpublished). Unless there are intraspecific differences, therefore, no Sitopsis species has a genotype which matches the activity of chromosome 5. Consequently, the control exercised by this chromosome probably developed after the inclusion of the B genome in polyploid wheat. If, as this suggests, the genetic regulation of the diploid-like behavior of wheat had a mutational origin, a number of hypotheses can be advanced concerning the nature of the change involved. It should be indicated, however, that a mutational origin of the system is most readily conceived if changes at a single locus are involved. Therefore, the succeeding hypotheses are founded on such a notion, although a more complex organization cannot be excluded.

The significance attached to the different meiotic patterns produced by *A. speltoides* and the other Sitopsis species, in hybrids with wheat, is critical in every evaluation of the problem. For example, it may be that the distinctive behavior of *A. speltoides* depends upon a gene in the chromosome homologous, or homoeologous, to chromosome 5 of wheat. Then the different effects on pairing produced by *A. speltoides, A. longissima* and chromosome 5 might depend upon different alleles at the same locus. Of these the *A. speltoides* allele would be dominant to the other two, and in terms of the others the *A. longissima* allele would be an amorph. On this hypothesis the chromosome 5 allele could have arisen from either of the others by mutation. However, a recessive mutation from the dominant is more probable, and this would fit with other evidence favoring the derivation of the B genome from *A. speltoides*.

Alternatively, *A. longissima* and *A. speltoides* may carry the same allele, at the locus

TABLE II. Mean chromosome pairing at metaphase I of meiosis in hybrids with and without wheat chromosome 5

Hybrid	Chrom. No.	cells	univ.	Bivalents rod	Bivalents ring	Bivalents total	triv.	quad.
T. aestivum × *A. speltoides*	28	50	6.04	4.60	2.04	6.64	1.88	0.76
T. aestivum × *A. speltoides*	28	50	3.40	4.14	2.00	6.14	2.20	1.38*
T. aestivum × *A. speltoides*	27	30	6.13	2.63	2.50	5.23	1.76	1.23†
T. aestivum × *A. speltoides*	27	30	6.90	3.37	2.73	6.10	1.33	0.93†
T. aestivum × *A. longissima*	28	100	23.90	1.96	----	1.96	----	----
T. aestivum × *A. longissima*	27	100	7.50	5.97	1.61	7.58	0.70	0.56
T. aestivum × *A. longissima*	27	100	9.80	5.54	0.87	6.45	0.87	0.41

*Associations of five, 0.04 per cell.
†Associations of five, 0.03 per cell.

concerned with meiotic pairing, on their chromosomes which correspond to chromosome 5 of wheat. Then the gene-differences, causing different meiotic pairing in hybrids, would reside elsewhere in their genotypes. If this were so, mutation on chromosome 5 might have caused it to assume control of intergenome pairing in the polyploid. This would presumably require no other alteration to a genotype like that of *A. longissima*. However, if the *A. speltoides* genotype were involved, the alteration of the epistatic genes, which suppress the chromosome 5 effect, would also be necessary.

Then again it might be that *A. speltoides* and *A. longissima* are indeed different at the significant locus of the chromosomes corresponding to wheat chromosome 5. On this hypothesis *A. speltoides* might already carry the same allele as wheat chromosome 5, but epistatic genes elsewhere in the genotype suppress its effects and cause the breakdown of homoeologous isolation in hybrids. Under these circumstances, if *A. speltoides* had contributed the B genome, mutation of the epistatic suppressors could have unmasked the chromosome 5 condition. It would then be left in control, exercising its restrictive influence on homoeologous pairing.

Synthetic tetraploids, derived from diploid wheat and either *A. speltoides, A. bicornis* or *A. longissima,* all form multivalents at meiosis, behaving more like segmental allopolyploids than true allopolyploids[3,5,9]. Presumably the first tetraploid wheat also behaved in this way, although all contemporary polyploids are bivalent formers. Consequently, there would be considerable selective advantage in favor of a change which reduced or eliminated homoeologous pairing, and restricted conjugation to homologous partners only. Selection would favor the purely bivalent formers because of their genetically balanced gametes and improved fertility. It seems likely that a mutation producing this result occurred either on chromosome 5 or elsewhere in the B genome, and subsequently the mutant was selected to fixation.

Since the pairing control exercised by the B genome of the polyploid wheats could have originated either from the *A. speltoides*- or from the *A. longissima*-type of organization, the present results give little assistance as to the source of that genome. However, it should be noticed that two of the three hypotheses proposed somewhat favor an origin from *A. speltoides*. As indicated earlier, the karyotype and gross morphology of this species also suggest it as the most likely source of the B genome.

Summary

Chromosome 5 of hexaploid common wheat, *Triticum aestivum*, carries one or more genes which prevent homoeologous chromosomes from pairing at meiosis. This chromosome is in the B genome and was probably derived from a species in the Sitopsis section of *Aegilops*. Consequently, the Sitopsis species may be examined to determine whether any have a genetic influence on meiotic pairing similar to that of chromosome 5. Chromosome pairing has been compared in hybrids between common wheat, with and without chromosome 5, and two Sitopsis species, *A. speltoides* and *A. longissima*.

Homoeologous pairing occurred in the hybrids involving *A. speltoides* irrespective of the presence or absence of chromosome 5. Therefore, this genotype removes the inhibition of wheat chromosome 5 on intergenome pairing.

There was no abnormal intergenome pairing in hybrids involving *A. longissima* when chromosome 5 of wheat was present. In its absence, however, the isolation between the genomes broke down. Thus, the *A. longissima* genotype is unable to compensate for the deficiency of chromosome 5 and cannot operate in the same way.

Apparently none of the possible B genome donors has a genotype which functions like the critical wheat chromosome. Therefore, a number of alternative hypotheses are proposed to account for the origin by mutation of the diploidizing influence exercised by chromosome 5.

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A GENETIC ANALYSIS OF THE DOUBLE CERVIX CONDITION IN CATTLE

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DURING a post-partum examination of the genital organs of the cows in the University of California herd of registered Herefords at Davis (UCD) in 1954, one of the authors (J. W. Kendrick) observed a considerably higher incidence of double cervices than usually found in dairy cattle examined in the Veterinary Clinic at Davis.

The double cervix condition is a congenital malformation of inhibitory nature in the cervical region of the female reproductive tract of some higher mammals and is manifested as a persistence of the median walls of the Müllerian ducts.

Observations

Examinations by the speculum method performed on almost all UCD breeding cows and yearling heifers in ensuing years showed 17 animals (12.8 percent) of 125 examined and classified to exhibit some degree of arrested Müllerian duct development in the cervical region.

Due to considerable inbreeding in the UCD herd, it was suspected that the high incidence of double cervices was genetically controlled. Therefore, an identical survey was initiated in the San Joaquin Experimental Range (SJER) herd of grade Herefords where, except for one Hereford and two Aberdeen Angus bulls, UCD bulls had been used exclusively for 20 years. Cervical malformations identical to those of the UCD herd were found in 19 of 279 animals (6.81 percent).

The high incidence of double cervices in two herds of similar breeding maintained under different environmental conditions provides evidence that the etiology of the defect is genetic, since no factor common to the two environments is apparent that could account for the higher frequency. Nongenetic modifying effects of some degree may be present, considering the length of time required for the development of the genital tract, but only positive observation of such environmental influences can be conclusive.

A comparison of the pedigrees of cows with abnormal cervices in the UCD herd showed these animals to be descendants of the bull Dandy Domino 46th (DD46), who also appears as a direct ancestor of all affected cows in the less inbred SJER herd. When the study of pedigrees was extended to include all cows classified according to cervical condition, it became apparent that the great majority of all normal females also traced back to this bull—most of them on both parental sides, as in the case of the affected females.

In order to evaluate the importance of hereditary factors in the manifestation of the defect it became necessary to enlarge the investigation and to include a third herd with a sufficiently different breeding history. The University of Nevada herd of registered Herefords (UN) at Reno met this condition, and in 1956 a total of 81 cows were examined. Only two of these cows traced back to DD46 on both parental sides and persistence of the median walls of the Müllerian ducts to any degree was observed in only one instance (1.23 percent).

The observations from the three herds and the types of relationship to bull DD46 are summarized in Table I. The totals for the UCD and SJER herds in-

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clude 10 Aberdeen Angus × Hereford crossbreds, one of which had a double cervix, and 18 backcrosses.

Analysis and Discussion

On the basis of the principle of genetic parsimony and the fact that the majority of hereditary anatomical abnormalities studied in cattle are recessive, the hypothesis of a recessive autosomal gene was investigated. This hypothesis is supported by the fact that 86 percent of the double cervix cows are direct descendants of DD46 on both parental sides. The relative lack of DD46 breeding in the UN herd, furthermore, is associated with a minimal incidence of double cervices in that herd when compared with the other two herds under study. Recessiveness is also strongly indicated by the fact that of the 37 double cervix cows found, at least 14 had single cervix offspring and/or normal full sibs.

In an attempt to account for the five double cervix cows that trace back to the common ancestor on one parental side only, one may consider the incidence of the defect found in other cattle populations. Reports on double cervices in beef cattle are very rare because of the virtual absence of relevant studies, but the defect is known to occur in Herefords[30, 31, 39]. In dairy cattle, on the other hand, double cervices are considered a not too uncommon abnormality by several authors in Europe and in the United States[27, 14, 30]. Spriggs[33] describes 10 cases and in a review article estimates the incidence of double cervices at 0.2 percent in the total cattle population in his district in Scotland, with a possible predisposition to the anomaly in British Friesians.

In a slaughter house survey of ovarian hypoplasia, Lagerlöf and Boyd[21] found 118 double cervices (two percent) in 5,950 cows, exclusively of the Swedish Highland breed of dairy cattle. The incidence in cows from one of the four counties was more than twice as high as that in cows from the other three counties. This investigation represents the largest sample of cows containing pertinent information.

For purposes of discussion, suppose one takes the incidence of double cervices in the general population to be two percent. On the assumption that double cervices are caused by a single recessive gene, a zygotic frequency of two percent indicates a gene frequency of 0.14 in the general population. In the present material double cervices were found in 37 of those 420 females that trace back to DD46 (Table I). This incidence of nine percent corresponds to a gene frequency of 0.24 for the population of individuals descending directly from DD46, considering an average inbreeding of seven percent and assuming no selection against either allele[42]. Using the gene frequency of 0.24 for the population of descendants of DD46 and the hypothetical gene frequency of 0.14 for the general population (i.e., animals not tracing to DD46) results in a probability of 0.034 for double cervix cows tracing to DD46 on one parental side only. This computation is given in Table II.

If one assumes further a Poisson distribution (with $p = .034$, $N = 72$) for the incidence of double cervices in the population of cows tracing to DD46 on one side only, then the probability of obtaining five or more abnormal cases is 0.10.

Of the 65 cows that were not direct descendants of DD46, none had a double cervix. The probability of this result, again using a zygotic frequency of two percent for the general population, is 0.27.

Table III summarizes the above considerations and includes, for comparison, expected values that are based on an incidence of double cervices of only one percent in the general population.

TABLE II. Zygotic frequencies expected among cows only one of whose parents traces to DD46 (based on the recessive gene hypothesis)

		Gametic ratios for parents tracing to DD46	
		$q = .76$	$p = .24$
Gametic ratios in the general population	$q = .86$	.654	.206
	$p = .14$	.106	.034

TABLE I. The frequencies of double cervices and of types of relationship to DD46 among cows in the three herds studied

No. of parents tracing to DD46	Herds											
	UCD			SJER			UN			All herds		
	SC*	DC†	Total	SC	DC	Total	SC	DC	Total	SC	DC	Total
0	1	0	1	1	0	1	63	0	63	65	0	65
1	28	2	30	24	2	26	15	1	16	67	5	72
2	79	15	94	235	17	252	2	0	2	316	32	348
Total	108	17	125	260	19	279	80	1	81	448	37	485

*Single cervix.
†Double cervix.

Testing the actually observed frequency of double cervices among cows tracing back to DD46 through both parents against expectation based on the hypothetical incidence of two percent in the general population results in a highly significant χ^2 value ($\chi^2 = 7.64$, $P = .006$.).

The above considerations indicate that the incidence of double cervices among animals tracing back to DD46 on one or neither parental side is consistent with the hypothesis of a single recessive gene. This conclusion is, of course, contingent upon the extent to which the range of frequencies of the trait in the general population (one to two percent) is realistic.

The relatively high value of 0.14 for the frequency of a gene causing an abnormality in a large population is not inconceivable in view of the fact that a double cervix probably exerts at most only a small adverse effect on reproductive efficiency and none at all on viability. Most authors are agreed that double cervices do not appreciably decrease fertility[4, 17, 22]. Spriggs[33] states that double cervices were present in less than one percent of the cows examined by him on account of infertility, while the incidence for all cattle in that region of Scotland was estimated at 0.2 percent. However, Gotink *et al.*[14] consider double cervices a serious defect in artificial insemination of dairy cattle with respect to conception rate. Bonfert and Mai[3] found a high percentage of cervicitis and sterility in double cervix cows and they advise that such cows be culled rather than remain in an artificial insemination program.

Reproductive efficiency may be adversely affected in two ways by the trait under consideration. First, penetration of the sperm into the uterus may be impaired, resulting in a lowered conception rate. Secondly, at parturition, the functional segment of a true or false double cervix may not dilate sufficiently to accommodate expulsion of the fetus, or the vaginal pillar behind a single cervix may interfere with normal delivery. Severe dystocia may be the result, possibly leading to injuries to the genital tract and to stillbirths.

Table IV summarizes the reproductive performance of single and double cervix cows in the UCD herd, which is under close observation during the breeding and calving seasons. This information is not available for the SJER herd.

Pasture breeding for at least three heat periods was the rule in the UCD herd, although a limited number of cows were inseminated artificially during the 1956 season as part of an outcrossing experiment. Of 125 cows and heifers classified with respect to the trait in question, 21 were never exposed to the bull because of young age or culling. The data are otherwise entirely unselected.

The high absolute number of abortions and stillbirths are due, respectively, to a leptospirosis infection of the herd in 1954 and to the experimental practice of having heifers calve for the first time as two-year-olds. Proportionately equal numbers of single and double cervix heifers calved at this early age. The number of live, stillborn, and aborted calves as a proportion of the number of seasons that the cows were exposed to the bull

TABLE III. Comparisons of observed with expected frequencies of the double cervix condition in two populations of cows; in one, cows trace to DD46 on only one side of their pedigree, while animals in the other are not descendants of DD46

			Hypothetical incidence of double cervices in the general population			
			2 percent		1 percent	
No. of parents tracing to DD46	No. of cows classified	Proportion of double cervices observed	Expected proportion of double cervices	Probability of results obtained	Expected proportion of double cervices	Probability of results obtained
0	65	0	.02	.27	.01	.53
1	72	.07	.03	.10	.02	.03

TABLE IV. Comparison of the reproductive efficiencies of single and double cervix cows

Type of cow	No.	Breeding seasons	Live calves	Stillbirths	Abortions	Parturitions assisted	Av. no. pregnancies/breeding season	Av. no. parturitions assisted/full-term pregnancy
SC	88	367	280	20	13	38	.85	.13
DC	16	71	56	3	2	10	.86	.17

is the only criterion possible for a comparison of conception rates. The respective χ^2 value is exceedingly small and the corresponding P value is 0.97.

Adverse effects of double cervices at parturition may be indicated by the number of times assistance was found necessary out of the total number of births terminating an approximately normal gestation period (Table IV). The χ^2 value for the difference between single and double cervix cows with respect to ease of delivery is again insignificant with a P value of 0.58. There is no case on record in the present material where difficulty at parturition was due to a double cervix.

Richter[27] and Scorgie and Ottaway[32] report instances in which a vaginal band interfered with parturition. In these cases no further assistance with delivery was necessary after the band was cut surgically. Richter *et al.*[28] review several analogous cases of dystocia, but these are very rare events.

The prominence of DD46 in the pedigrees of single as well as double cervix cows in the UCD and SJER herds is mainly due to the very extensive use of bull 680, a grandson of DD46. Bull 680 was considered so outstanding that the breeding program for the UCD and SJER herds was designed to build up the relationship of the herd to him and to maintain this through the use of his sons and grandsons in subsequent generations. It was 680 and his immediate offspring that were the parents of the majority of the cows with double cervices in the two herds.

Among the pedigrees of the animals directly concerned with the anomaly, three pertinent types of matings may be distinguished. In these matings bulls deemed heterozygous by progeny test results (on the basis of the recessive gene hypothesis) were mated to double cervix cows (type A), to single cervix cows with double cervix offspring (type B), or to unclassified cows with double cervix offspring (type C).

Discarding the first double cervix offspring of each cow of types B and C and of each bull as identifying the particular animal as heterozygous, a summary of test-mating results was compiled and is presented in Table V. The P values are based on the assumption that the double cervix condition is caused by a single autosomal recessive gene. All cows of type C and the bulls that sired the double cervix animals are considered heterozygous for the gene.

On the basis of the test matings between heterozygotes (types B and C) the 3:1 hypothesis is not rejected. The results of both types of test matings are deficient, but in the same direction. The relative lack of double cervix offspring may be partly due to the fact that 18 of the 35 single cervix cows concerned were classified after having produced a total of 45 calves. At parturition a relatively thin vaginal band may have been severed and may thus easily have escaped detection during subsequent examinations. In two double cervix cows such bands were found to have been ruptured during the fourth and sixth parturition, respectively. While Hanson[17] considers vaginal bands the most common of all genital defects in cattle, they were found in the present material with a much lower frequency than incomplete double cervices.

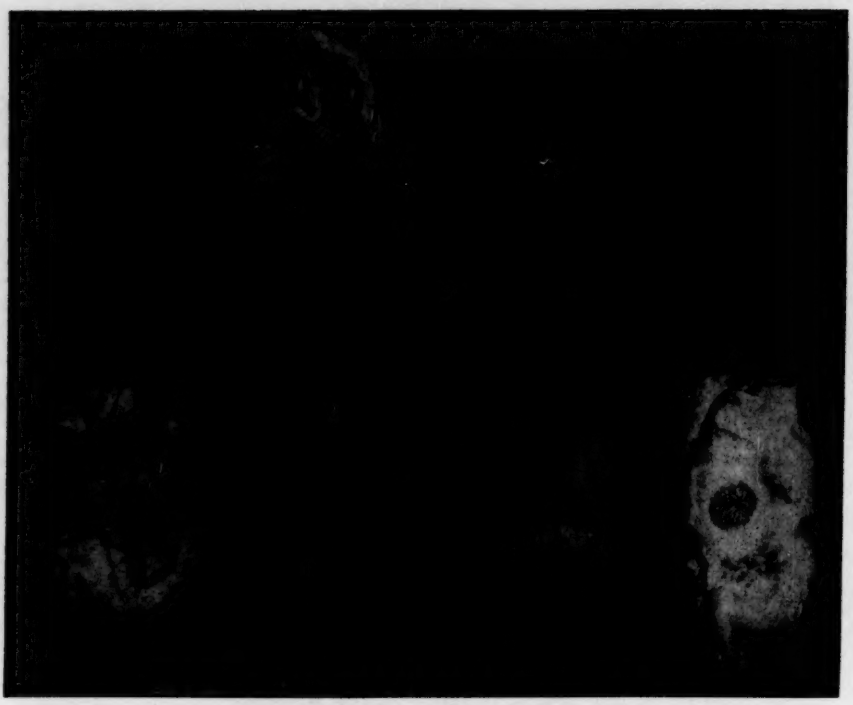
Low penetrance of the gene for double cervices may be advanced as the major explanation for the deficiencies of the mutant types in the observed results of both types of test matings. In order to develop this point it is necessary to consider the anatomy of the female reproductive tract in several species of mammals.

In mammals the reproductive system is the last group of organs to be formed in the embryo and it is the most primitive system at the time of birth[41]. In females it is particularly prone to anatomical defects which are then frequently of a congenital nature. Many of these defects are due to deficient development of the Müllerian ducts which, in mammals, give rise to almost the entire tubular portion of the female genital tract.

Excepting monotremes and lower marsupials, all mammals have a single vagina and differences in the anatomy of the female genital organs of different species of mammals depend largely upon the extent of fusion of the Müllerian ducts, especially of their uterine segment. In primates fusion of this segment is complete, resulting in a uterus simplex, whereas in ruminants and horses the anterior part remains separate, giving rise to a uterus bicornis. Union of the uterine segment of

TABLE V. Results of test matings

Type of mating	UCD		SJER		Both herds		Expected ratio		P values
	SC	DC	SC	DC	SC	DC	SC	DC	
A	5	1	7	0	12	1	1	1	.0017
B	10	2	5	2	15	4			
C	2	0	6	0	8	0			
B+C	12	2	11	2	23	4	3	1	.1583
Total	17	3	18	2	35	5			



INCOMPLETE DOUBLE CERVICES

Figure 9

A and *B* show two ora uteri externa separated at the posterior end by cervical and vaginal tissue, respectively. *C* shows transverse sections of the functional segment (on the right) and the place of junction with the non-functional segment in the second section.

the Müllerian ducts is still less complete in carnivora and swine which have a uterus bipartitus with a common cervical canal and a very short undivided uterine body. In rodents fusion in the uterine region proceeds to the least extent and a uterus duplex (uterus bicornis et bicollis) with two cervices and two uteri is normally found[43].

On rare occasions congenital malformation of Müllerian duct derivatives which resemble anatomical differences existing between species are observed within certain species of livestock.

A variety of defects may be caused by segmental aplasia and arrested development of the Müllerian ducts, primarily in the region of the uterine horns, uterus, and cervix, while the oviducts and the anterior part of the vagina are in most instances essentially normal. This report deals specifically with the persistence of the median walls of the Müllerian ducts in cattle, where lack of complete fusion of these ducts is found more frequently than other congenital abnormalities of the tubular tract. A uterus didelphys is the most severe and least frequently observed stage of inhibited fusion in cattle and only very sporadic occurrences are known[1, 7, 8].

Investigating the embryological development of the female genital tract in cattle, Bissonnette[2] found that in the 51 mm. embryo the Müllerian ducts unite to form a single lumen in the uterine and vaginal regions, while in the intermediate region of the future cervix they remain separate and are still present as two distinct canals in the 75 mm. embryo. In view of this lag in fusion, it is not surprising that the cervical region is more apt to exhibit persisting median walls than are the other two regions. It is convenient to assign such cervical vestiges to one of three classes:

1) *Complete or true double cervix.* There are two separate cervical canals interconnecting the vagina and an undivided uterus, as described in South African Friesians[26],

in cattle of northern Italy[38], in Swedish Highland cattle[21], and in Egyptian buffaloes[16].

2) *Incomplete or false double cervix.* The caudal part of the cervix is involved, with the cervical canals leading from two distinct ora uteri externa joining anywhere up to halfway through the cervix. However, early embryonic growth may be somewhat asymmetrical and one of the cervical openings may end in a blind pouch[14].

3) *Vaginal septum.* Fusion of the Müllerian ducts is incomplete at the caudal end of the cervix causing the retention of a vertical pillar or of a septum of varying size and shape. In ruminants these vaginal septa are considered to be of the same persisting structure as the uterine septum[43]. Basically composed of connective tissue covered with vaginal epithelium[24], the septa adhere to the roof and floor of the anterior vagina behind a single cervix and extend caudally up to 15 cm.

Such fibrous vaginal bands and incomplete double cervices (classes 2 and 3) are well known to veterinary practitioners in many countries. They have been recorded in British, Swedish, German, and South African Friesians, Holsteins, Jerseys, Guernseys, Shorthorns, Red Danish dairy cattle, Swedish Highland cattle, Brown Swiss, Simmenthaler, and the Egyptian buffalo[3,10,16,18,19,21,26,30,33,36,38].

Vaginal septa may also be encountered in combination with bicornuate uteri[6,35] and quite often with true and false double cervices, but they are to be distinguished from a persistent hymen which is an unrelated condition. A rare form of an incomplete double cervix was observed by Wheat[39] when he examined a Hereford cow that was a paternal half sister of several double cervix cows in the present set of data. The right, nonfunctional segment of this incomplete double cervix was bent outward at a right angle and ended in a blind pouch; furthermore, the thin septum between the two segments continued into the vagina where it widened and assumed a triangular shape almost occluding the two openings.

No congenital defects of the female reproductive tract, other than the cervical abnormalities mentioned, were observed in any of the three herds during this investigation. *In vivo* observations and autopsies of the 37 affected animals showed that the vast majority of defects were in the form of an incomplete double cervix (Figure 9). Several animals had a vaginal pillar or septum behind a single cervix, and only one complete double cervix was found. A more accurate classification is not possible, since a number of the cows concerned are still alive and not all of those disposed of could be followed through slaughter and dissected subsequently. In only one abnormal cervix, however, did the doubling not conform to the usual pattern, in that the cervical canal on the right side protruded for a considerably longer distance into the vagina than did the left duct. A certain amount of left-right asymmetry may be expected, because the two Müllerian ducts increase in length at different rates in the 24 to 27 mm. embryo[2].

With incomplete double cervices, one of the separate segments usually has a larger diameter and appears patent and functional, but during pregnancies both openings may be closed by a cervical seal.

A large set of anatomical aberrations of the tubular tract, usually associated with white coat color, is collectively referred to as white heifer disease. The abnormalities classified under this term range from total aplasia of Müllerian duct derivatives to a constricted hymen as the only defect[34], and sterility often ensues. Despite occasional overlaps in manifestation, persistence of two Müllerian ducts in all or a part of the cervical region differs from the "typical" white heifer disease, in that development of the genital tract of afflicted white heifers is usually arrested much earlier and inhibition of growth thus lasts for a longer period of time. The same holds for the gross malformations described by Fincher and Williams[11]. White heifer disease is by no means well defined and a more concise statement of the relationship to the double cervix condition therefore is not possible.

Furthermore, vaginal bands and a complete double cervix occurred in association with an obliteration of the vagina, a new kind of genital malformation found by Nordlund[25] in 12 daughters of one bull.

Cervical defects of a congenital nature are encountered in cattle more frequently than in other species of livestock[32]. In sheep anomalies of the internal genitalia are uncommon[20], but at least two pertinent cases are on record. Curson and Belonje[5] described an incomplete double cervix in a Merino ewe, in which only the os uteri internum was patent; the two posterior openings were closed and great dilatation of the cervix was associated with prenatal death of twin embryos. Dutt[9], furthermore, found two cervices and a septum in the anterior vagina in one ewe out of a lot of 90 crossbred, dark-faced, northwestern ewes that were slaughtered in Kentucky. Congenital defects of the reproductive system are also rare in mares[30]. Macrae[23], however, recorded one instance in which a Clydesdale mare had an incomplete double cervix analogous to those found in cows.

Large-scale surveys of the genitalia of sows and gilts have been carried out at slaughter houses in several countries. Wiggins *et al.*[40] report an incidence of 0.04 percent of double cervices in 5,088 slaughter hogs, in addition to other manifestations of defective development of the Müllerian ducts which are of the same type as those known in cattle. A septum or string was present in the anterior or

posterior vagina in 1.446 percent of 9,250 gilts and in 0 percent of 476 sows examined in Denmark[37]. In contrast to cattle, vaginal septa in hogs occur mainly near the introitus vaginae and extend cranially[15].

Earlier mention of vertical bands across the os uteri externum is made by Cartwright[13] who found these structures to cause dystocia in a cow and a sow.

Although many of the Asiatic and African breeds have not been examined, it can be safely assumed that double cervices occur in all of the economically important breeds of cattle which have been examined and to a variable extent also in related species and families of livestock.

Mutations causing anatomical defects, which, like double cervices, occur in several families of the order Ungulates and which resemble a more primitive structural pattern common to lower mammals, may conceivably have been occurring in the order Ungulates since its beginnings[12]. If this is true in the case of double cervices, natural selection may have been active for many generations and may have changed the mode of expression of the gene from original dominance in the lower mammals to complete recessiveness in present-day members of the higher families concerned. Although the selection pressure against the gene associated with the more primitive condition must have been slight, the change in relative gene action could have been accomplished by the accumulation of modifying genes during the evolution of the higher families. According to current evolutionary theory this process is expected to result in an irregular manifestation of genes causing the less rare defects, including total absence of the respective defect within the limits of standard methods of detection[29].

Modifying gene action thus tends to decrease the penetrance of the more common harmful genes, and it influences the degree of severity of expression. Expressivity in the present observations ranges from a thin vaginal pillar to a complete double cervix, with the less severe forms occurring much more often.

It should be mentioned also that Gotink *et al.*[14] strongly considered the possibility of genetic constitution in the etiology of double cervices, and Scorgie *et al.*[32] more specifically suggested that the abnormal character is transmitted as a recessive. Bonfert *et al.*[3] likewise suggested a recessive mode of inheritance; among a total of 12,144 Black Pied Lowland cows at an artificial insemination center, they encountered 43 cows with a double os uteri externum, 20 of which were sired by five bulls.

Summary

An excessively high incidence of the double cervix condition in two closely related herds of Hereford cattle is reported. The evidence indicates that inheritance of this sex-limited defect is conditioned by a single autosomal recessive gene with low penetrance and variable expressivity. Double cervices seem to occur in many domestic breeds of the bovine species and they have also been observed in other classes of livestock. The adverse effect of the double cervix condition on fertility in Hereford cows is extremely small under ordinary beef cattle management.

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A SPONTANEOUS MOSAIC IN DROSOPHILA

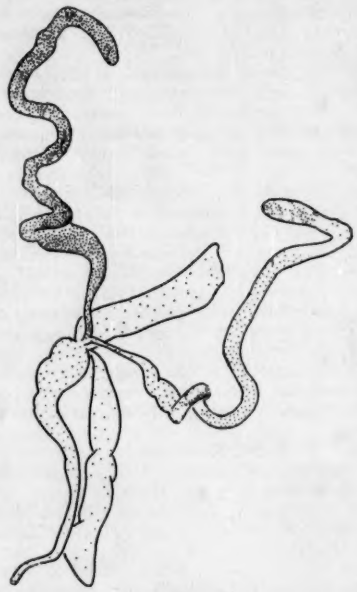
STANLEY KATZ*

AUERBACH[1] has made a comparison in *Drosophila* of the mosaic individuals resulting from exposure to X-rays with those induced by chemical mutagens. The X-ray induced mosaics found in *Drosophila* are rarely gonadic mosaics, the gonads generally consisting of either mutated or non-mutated cells. This was not the case among the mustard gas mosaics studied by Auerbach[1] in which instances of gonadic mosaicism were frequent, as shown by the presence of both mutated and non-mutated regions in the gonads. A spontaneous mosaic resembling the latter type was encountered during a series of routine crosses, and this report presents a morphological description, as well as data on the breeding tests, of this spontaneous mosaic.

Materials and Methods

The mosaic male appeared in the F_1 generation of a cross between a wild-type female (*Ore-R*) and a mutant *rucuca* male (*ru h th st cu sr e^s ca*). The right eye of the mosaic male was completely white while the left eye contained a sector of white in a red field (Figure 11). The right ocellus was white, that on the left was red, and the median ocellus was white on the right side and red on the left. In addition, two dorsocentral bristles on the left side of the thorax were missing.

In order to determine the possible extension of mosaicism to the gonads, the gametes of this mosaic male were analyzed by a series of breeding experiments. The male was mated to three groups of attached-X females, with matings spaced at six-day intervals. The progeny resulting from each mating were examined and scored for seven days after the initial date of hatching. White-eyed and red-eyed males were obtained in the F_1 generation while the females were all classified as attached-X. The data from these crosses are given in Table I.



TESTES OF MOSAIC INDIVIDUAL

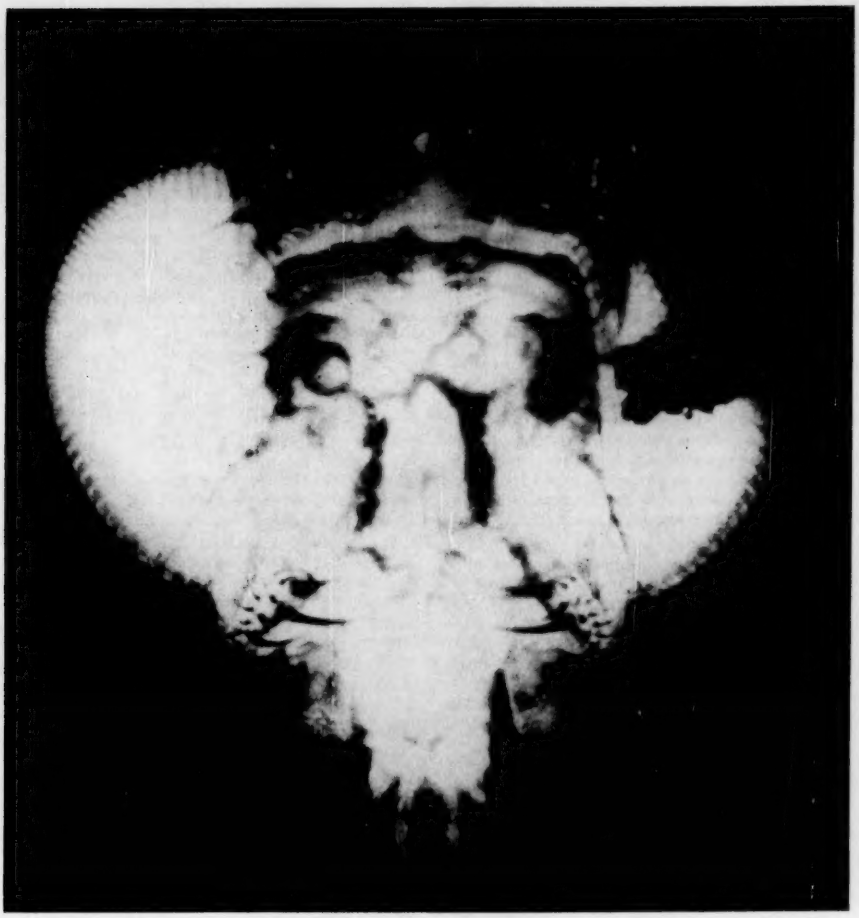
Figure 10

The dense stippling represents the pigmented areas of the testis sheath.

Results

The presence of two classes of males

*Department of Biology, Reed College, Portland, Oregon. I would like to express my appreciation to Dr. M. T. M. Rizki for his interest and suggestions throughout this project, and most particularly for his assistance in dissection of the reproductive system of the mosaic individual.



HEAD OF MOSAIC MALE

Figure 11

The mosaic effect is apparent in the median ocellus in the mid-dorsal region as well as the left eye.

with respect to eye color in these crosses indicates that the mosaic male parent was also mosaic for its gamete population, i.e., some germ cells carried the mutation for white eye on their X-chromosomes whereas the other gametes transmitted the normal allele.

It is interesting that other gene loci on the X-chromosome must have mutated also. The phenotype of the mosaic individual included the absence of two dorsocentral bristles, also absent in the

TABLE I. Progeny from crosses XXY yellow ♀♀ × *rucuca* + mosaic ♂

	Males			Females
	white eye	normal eye	total	XXY yellow
Mating I	28	97	125	128
Mating II	26	60	86	72
Mating III	43	132	175	151
Totals	97	289	386	351

F_1 males. Unfortunately the variation in the bristle characteristics was not scored during the course of the experiment; however, it was noted that the bristle character was independent of eye color and was associated with some of the red-eyed males as well as some of the white-eyed males. It did not appear in any of the female offspring.

Dissection of the mosaic male was performed after the breeding experiments were completed. The testes were removed and examination revealed the mosaic nature of the pigmented sheath (Figure 10).

Discussion

The spontaneous occurrence of a mosaic individual in which mosaicism extends to the gonadal tissues and the gametes presents an interesting dilemma as to the nature of its origin. In this case at least two independent mutations on the X-chromosome have taken place, white eye and the bristle character. The existence of all four types of males in the F_1 generation, that is, white-eyed males and red-eyed males each with and without dorsocentral bristles, can be explained if one assumes that in some cells the mutation in the X-chromosome affected the white locus, in other cells the bristle locus was affected, and in still other cells both loci were altered by mutation.

Crossing-over cannot be offered as an explanation in this case in the *Drosophila* male. It seems that during the early development of this zygote, a transient physico-chemical environment may have lasted long enough to bring the hereditary material in a metastable state so that mutations took place in more than one X-chromosome. However, these results could also be explained on the basis of Auerbach's hypothesis of delayed effect due to a sorting out of mutated and nonmutated subunits of a single affected chromosome.

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CASTOR BEAN SEEDLINGS AS A TEACHING AID

Hava Stein*

TWO seedling color characters recently found in the castor bean (*Ricinus communis*), and to be fully described elsewhere, may prove suitable for the demonstration of dihybrid segregation and gene interaction. Both characters are conspicuous, and since chlorophyll is not affected, all segregants are fully viable.

Approximately 100 seeds may be planted in a 20″ × 14″ greenhouse flat; at 77° they germinate within 10 to 14 days, and may be classified as soon as the cotyledons are fully spread. The castor bean plant is perennial. Hence F_1 plants may be grown in frost-free areas for four to five years, and will yield a continuous crop of F_2 seeds, provided only that proper isolation from other castors, ornamental or wild, is maintained. It is hoped that this laboratory will soon be able to supply those interested with limited amounts of F_1 and F_2 seeds.

Dominant *M* (mahogany) is known to de-

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termine the presence of anthocyanin in most aerial plant organs[1] including the hypocotyl. Recessive *m* (green) limits anthocyanin distribution to pistils, young leaves, and the hypocotyl. Due to the presence of anthocyanin in the hypocotyl of both *M* and *m* plants, these types cannot be distinguished at the seedling stage. A new gene, m^p (pure green), which is the most recessive allele at this locus, is responsible for the absence of anthocyanin from all plant parts, including the hypocotyl[3]. (Some lines carrying m^p do contain anthocyanin in the seed coat; this, however, is not relevant to the present discussion.) Therefore, segregating populations may be easily classified into *M* and m^p plants at emergence.

Recessive *ag* (anthocyanin glands) acts as a minor color gene. It imparts a deep purple color to all organs of *M* plants, and in m^p plants, it is responsible for the appearance of red dots and stripes on stems and veins. However, its main effect in *M* plants is the appearance of fine red dots on both surfaces of the cotyledons (as well as on all true leaves). These dots, which are either greatly distended single epidermal cells or pigment-filled subepidermal spaces, are clearly visible to the naked eye because of their intense red pigmentation. In *MAg* plants, similar dots, though smaller and paler, are found too, but are limited to the lower side of the cotyledon. Hence, the earliest and most clear-cut diagnostic character for *Mag* plants is the presence of red dots on the upper surface of the cotyledon. In populations segregating for *M* and *ag*, *M* seedlings can thus easily be separated into *Mag* and *MAg* groups, while among m^p plants, m^pag and m^pAg cannot be separated until about three weeks after emergence. At that time, red dots appear on the nodes of m^pag plants. Therefore, all four classes of segregants—*MAg*, *Mag*, m^pAg, m^pag—cannot be demonstrated at the same stage. However, after the ratio of *M* and m^p plants has been determined, as well as the ratio of *Mag* and *MAg* plants within the *M* class, all *M* seedlings may be removed from the flat. This will afford sufficient space for the remaining m^p plants to grow until ready for classification. Alternatively, the m^p and *ag* genes may, of course, be used simply to demonstrate a 9:3:4 ratio.

The phenotypes may be summarized as follows:

MAg—red hypocotyl, no dots on upper side of cotyledon.

Mag—red hypocotyl, red dots on upper side of cotyledon.

m^pAg—green hypocotyl, no trace of anthocyanin visible throughout life.

m^pag—green hypocotyl, red dots appearing at nodes within three weeks after emergence.

The segregations in Table I may serve as examples.

A third new gene which affects the seedling is *n*—noncolored. Its main effect is partial suppression of chlorophyll in sepals and capsules[3]. In green m^p and *m* plants, chlorophyll deficient capsules are ivory white. In anthocyanin-containing *M* plants, which ordinarily bear capsules of a reddish-brown color (which results from the superimposition of anthocyanin in the epidermis on chlorophyll in the mesophyll), chlorophyll deficient capsules appear bright pink or red. Seedlings are affected to a lesser extent and cotyledons are greenish yellow, particularly at the base of the lamina; seedling vigor seems unimpaired. *N* and *n* types do not contrast as sharply as different anthocyanin types, and the seedlings must be observed at wider spacing and in full light, i.e., they must be grown in the open. As the difference is transient (true leaves and stems show hardly any signs of chlorophyll deficiency, and cotyledons of both types eventually lose their chlorophyll), classification is only about 85 percent to 90 percent correct (as checked against classification by capsule color). Still, the *n* gene may have its use in the demonstration of trihybrid ratios, where the required populations are in any case too large to be accommodated indoors (Table II).

TABLE I. Segregations for genes *ag* and m^p

	MAg	*Mag*	m^pAg	m^pag	χ^2
$MMagag \times m^pm^pAgAg$, F_2	73	19	26	5	0.498
$Mm^pAgag \times m^pm^pAgAg$, BC_1	23		21		0.090
$Mm^pAgag \times MMagag$, BC_1	109	89			2.020

TABLE II. Trihybrid ratio showing segregation of *n as recorded by capsule color (*mn*—white capsule, *Mn*—pink capsule)**

	MAgN	*MAgn*	*MagN*	*mAgN*	*Magn*	*mAgn*	*Magn*	*magn*
Observed	83	25	31	30	11	6	15	0
Expected	85	28.4	28.4	28.4	9.5	9.5	9.5	3.1
		$\chi^2 = 8.5927$, $\chi^2_{0.05}$ d.f.7 $= 14.067$						
		$P = 0.27$						

*Segregation ratio for *n* summarized over all anthocyanin types in $159N: 42n (\chi^2 = 1.7038)$.

In the same population, 182 seedlings (excluding very late emerging ones) had been individually marked for *n* as judged by cotyledon color. When checked against capsule color, 160 plants, or 88 percent of the population, proved to be correctly identified, eight were errors, and 14 had been marked "uncertain." If seedlings had been assigned at random to *N* and *n* classes in a 3:1 ratio, only 62.5 percent (¾ • ¾ + ¼ • ¼), or 114 plants in this population, would be expected to be correctly identified. The difference between the actual and expected identification percentages is highly significant, which proves that *n* affects the cotyledon, though not very distinctly.

The m^p gene was first observed in line 430/50 (also referred to as "Pure Green"). The line was started from five uniform plants grown from a commercial seed sample from Ecuador. These plants were selfed for five generations, and when they proved to breed true to all morphological characters, they were massed. Later, the gene was identified in three other lines of independent origin.

The *ag* gene was first observed in line 50/50 (also referred to as "Pure Red"). This line was started from seed of a single wild plant, supplied by the Centro Investigaciones Agronomicas, Maracay, Venezuela. It has been selfed for four generations, and was found to breed true to all morphological characters except partial spininess. The *ag* gene has since been identified in several ornamental castor varieties. It may well be identical with the *g* gene described by Harland[1] and Peat[2], but for *g* no information as to anthocyanin glands is available.

The *n* gene was first observed in line 126/50, which was started from a seed sample obtained from the United States. After one generation of inbreeding, the line segregated for capsule color, spininess, and sex type. From the white-capsule segregants, two true breeding lines were developed after three further generations of inbreeding. Both are monoecious, the line used in the quoted cross being spiny, while the other is spineless.

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THE INHERITANCE OF DIAPAUSE IN GRASSHOPPER EGGS

ELEANOR H. SLIFER AND ROBERT L. KING*

ALTHOUGH diapause in the eggs of various species of Acrididae has been studied extensively by physiologists[5], very little has been published concerning the inheritance of the phenomenon in this group. The most extensive and informative reports are those of Le Berre[2, 3, 4] on the isolation of stocks of *Locusta migratoria gallica* Remaudière and related subspecies in which eggs without diapause and eggs with diapause are produced. In earlier papers[10, 15] brief reference has been made to selection experiments which indicate that diapause, or its absence, in the eggs of the grasshopper, *Melanoplus differentialis* (Thomas), is inherited and is almost certainly under the control of multiple factors. The data on which this conclusion is based are presented below.

The experiments reported here were begun in 1928 when it was found that an egg pod laid by a female *Melanoplus differentialis* sometimes contains two types of eggs. When incubated at 25°C. a few eggs hatch on or close to the thirty-eighth day while the others go into diapause on the twentieth or twenty-first day[8]†. The eggs which enter diapause can be made to resume development by exposing them to lower temperatures for several months and then returning them to 25°C. Diapause eggs not treated with cold either hatch at irregular intervals over a long period or die. After treatment with cold the eggs in which diapause has been broken hatch in about 18 days. The total period, then, during which active development occurs is about 38 days, both in eggs with and without a diapause.

Materials and Methods

The source of the grasshoppers studied will be given under the separate headings below. The method used for maintaining stocks in the laboratory was, in general, that described earlier[8]. The food consisted of lettuce, bran middlings and dandelion (fresh in the summer months, dried in the winter). A supply of water was kept in the cages. The dishes of moist sand in which the eggs were laid were examined daily and the pods transferred to clean, damp sand to incubate at 25°C. When 28 days old, each pod was opened in water, debris removed and the eggs counted. At this stage of development — 10 days before eggs without diapause are due to hatch — it is usually easy to separate eggs which are developing from those which have entered diapause by examination with the dissecting microscope. The eggs, in a glass dish‡ and covered with water, are examined with a strong light (100 watt) placed near the table top so that light enters the dish from the side. With the light properly placed it is easy to locate the embryo inside most intact eggs through the semi-opaque chorion. The pigmented eyes of the embryos in diapause lie close to the posterior (micropylar) pole of the egg. If the egg has not entered diapause and development is continuing, the eyes will

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†Carothers[1] had noted earlier that the eggs of this species sometimes hatch without passing through a period of quiescence.

‡The dishes used are shaped like Syracuse watch glasses but are of heavier construction. The glass in the bottom is about one centimeter thick. Intact eggs are examined against a white background below the dish and eggs from which the chorion has been removed against a black background.

be found near the anterior end of the egg. In some eggs it is difficult to decide whether an embryo is present or the egg has spoiled. In all of the earlier work every such egg was dissected in Ringer's solution and its condition determined. Needless to say, this is a tedious and time-consuming task. The discovery[9] that the chorion of the egg could be removed by brief treatment with NaOCl without causing any injury made this part of the work much easier. Since that time all eggs which could not be classified readily and with certainty have been treated for a minute or two with NaOCl and then washed thoroughly in water. With the chorion dissolved away the egg contents are clearly visible through the transparent chitinous cuticle in which the embryo and yolk are still enclosed, so there is no need for dissection.

Results

1. Incidence of eggs with no diapause in wild stock

In 1947 and again in 1958 a series of egg pods—100 in all—was examined in order to determine the percentage of eggs which hatch without diapause, in pods laid by females caught in the field and brought into the laboratory. All of the grasshoppers were taken in September or October near Iowa City and so belonged to the subspecies *differentialis* recognized by Roberts in 1942. The results may be seen in Table I. Eighty-eight of the pods contained only diapause eggs, and of the remaining 12 pods, five contained one egg with no diapause, four contained two, one contained six, one seven and the remaining pod had in it 25. Of the 4206 viable eggs produced, 51, or 1.2 percent, developed without a diapause. If the single pod with 25 no diapause eggs is excluded, the percentage of eggs without diapause falls to 0.6. The high percentage of spoiled eggs in the 1947 series should be noted. Spoiled eggs in the 1958 series were not counted but the number, again, was high. The large proportion of acridid eggs which fail to complete development has been reported by other workers[3].

2. Selection for development without diapause (1928-1932)

The grasshoppers with which the first experiments were carried out were collected along the Delaware River, south of Philadelphia, in September 1928.

TABLE I. Occurrence of eggs which hatch without diapause in pods laid by females collected in the field

Year	No. egg pods	Mean no. viable eggs/pod	No. eggs with no diapause	No. eggs with diapause	Percent viable eggs with no diapause	Total no. eggs	Percent spoiled eggs
1947	54	49	51	2615	1.9	5600	52.2
1958	46	33	0	1540	0.0	—	—
Totals	100	42	51	4155	1.2		

TABLE II. Seven generations of selection for eggs without diapause (1928-1932). See Tables III and IV for single pair matings in sixth and seventh generations

Gen.	No. egg pods	Mean no. viable eggs/pod	No. eggs with no diapause	No. eggs with diapause	Percent viable eggs with no diapause	Total no. eggs	Percent spoiled eggs
1	48	—	—	—	—	—	—
2	26	106	458	2304	16.6	—	—
3	74	—	—	—	—	—	—
4	26	55	802	615	56.6	1934	26.7
5	17	83	1074	338	76.1	1680	16.0
6	20	47	334	599	35.8	1388	32.8
7	25	49	978	247	79.8	1891	35.2

These belonged to the subspecies *nigricans* later recognized by Roberts[7] as occurring in this area. Forty-eight egg pods were laid in the laboratory, and of these, five contained a few eggs which hatched about the thirty-eighth day at 25°C. These grasshoppers, which had hatched without passing through diapause, were isolated and used to start a line in which only individuals of this kind were saved to produce the next generation. At first all grasshoppers which hatched without a diapause were used, but in the fourth generation only those pods with more than 50 percent of the desired type of egg were saved, and in the fifth only those with 90 percent were kept to carry on the stock. During the first five generations the stock was handled as a mass culture, but in the sixth generation seven single pair matings were made. Two of these produced no eggs and the records of the other five pairs are shown in Table III. The offspring of pair 5, which produced 100 percent eggs with no diapause, were used to carry on the stock. Seven single pair matings in the seventh generation gave the results shown in Table IV. The highest percentage of eggs with no diapause produced by a pair in this generation was 97.1 from pair 3 and the percentage for all of the matings combined was 79.8. The stock, at this time, was vigorous and in excellent condition but was lost accidentally when it became infected with nematodes. The results for the seven generations of selection are summarized in Table II. The conclusions which may be drawn are (1) that the incidence of no diapause eggs may be greatly increased by selection; (2) that the offspring of a pair which has produced only eggs with no diapause may, themselves, produce eggs with a diapause; (3) that selection for eggs with no diapause does not lead to a decline in the viability of the stock; and (4) that the type of inheritance involved is complex.

3. Selection for development with diapause (1943-1950)

The 22 egg pods with which this series of experiments was begun in 1943 were taken from the stock of *Melanoplus differentialis* maintained in this laboratory

TABLE III. Results of five single pair matings in sixth generation of selection for eggs without diapause. See Table II for summary

Pair	No. egg pods	Mean no. viable eggs/pod	No. eggs with no diapause	No. eggs with diapause	Percent viable eggs with no diapause	Total no. eggs	Percent spoiled eggs
1	2	68	75	61	55.1	153	11.1
2	3	58	31	142	17.9	190	8.9
3	4	26	65	39	62.5	322	67.7
4	9	51	102	357	22.2	645	29.0
5	2	31	61	0	100.0	78	21.8

TABLE IV. Results of seven single pair matings in seventh generation of selection for eggs without diapause. See Table II for summary

Pair	No. egg pods	Mean no. viable eggs/pod	No. eggs with no diapause	No. eggs with diapause	Percent viable eggs with no diapause	Total no. eggs	Percent spoiled eggs
1	7	61	326	102	76.2	548	21.9
2	2	57	94	19	83.2	218	48.2
3	4	44	169	5	97.1	320	45.6
4	3	35	58	48	54.7	144	26.4
5	1	23	18	5	78.3	35	34.3
6	5	54	225	45	83.3	356	24.2
7	3	37	88	23	79.3	270	59.0

for many years. Since this colony had been started with grasshoppers collected in eastern Iowa, it belonged to the subspecies *differentialis* as limited by Roberts[7]. In order to start the series with eggs in which diapause was already well established, the eggs, after being incubated at 25°C. for four weeks, were placed in the refrigerator (5 to 10°C.) for four weeks. They were then returned to 25°C. for one week and all eggs in which diapause had been broken were discarded. Those still in diapause were treated with cold for four weeks longer and then transferred to 25°C. for another week. All those developing were discarded and the remainder returned to the refrigerator for a third period of four weeks. After one week more at 25°C. those eggs in which diapause had been broken by 12 weeks' exposure to cold (but not by eight weeks') were used to start the new stock. In the next generation the same type of selection was repeated but the time of exposure to cold was increased to 16 weeks and in the third generation to 20 weeks. In later generations the stock was carried on without selection by cold treatment of the type just described and egg pods containing large numbers of viable eggs were chosen to furnish parents for the next generation. During several generations diapause was broken by treatment of the eggs with xylol[10]. This eliminates the long wait necessary when cold treatment is used for this purpose. One-half hour of exposure to xylol replaces months of cold treatment. In other generations, whenever it was convenient to do so, the eggs were left in the refrigerator over the summer and hatched in September or October.

The results of nine generations of se-

TABLE V. Nine generations of selection for eggs with diapause (1943-1950)

Gen.	No. egg pods	Mean no. viable eggs/pod	No. eggs with no diapause	No. eggs with diapause	Percent viable eggs with diapause	Total no. eggs	Percent spoiled eggs
1	22	----	—	---	-----	----	----
2	2	115	0	230	100.0	237	3.0
3	10	103	0	1031	100.0	1238	16.7
4	2	112	0	223	100.0	279	20.1
5	13	63	0	813	100.0	1321	38.4
6	11	27	0	295	100.0	639	53.8
7	14	53	0	737	100.0	1221	39.6
8	14	48	0	674	100.0	1050	35.8
9	5	42	0	208	100.0	231	10.0

TABLE VI. Twelve generations of selection for eggs without diapause (1953-1960)

Gen.	No. egg pods	Mean no. viable eggs/pod	No. eggs with no diapause	No. eggs with diapause	Percent viable eggs with no diapause	Total no. eggs	Percent spoiled eggs
1	84	54	75	4448	1.7	6826	33.7
2	15	46	66	619	9.6	1272	46.1
3	35	37	72	1225	5.6	3856	66.4
4	43	38	744	883	45.7	4390	62.9
5	---	---	449	170	72.5	----	----
6	37	42	949	506	65.2	3692	67.8
7	290	32	3062	6148	33.2	----	----
8	201	31	2963	3249	47.7	----	----
9	115	38	1813	2536	41.7	----	----
10	35	38	714	627	53.2	----	----
11	23	28	452	198	69.5	----	----
12	8	17	81	56	59.1	1001	86.3

lection for a stock which produces only diapause eggs may be seen in Table V. Of the 4,211 viable eggs laid by this stock none hatched without a diapause. The conclusions which may be drawn from this set of experiments are: (1) that a true-breeding diapause stock can be secured by selection with relative ease and (2) that selection for the character is not accompanied by any loss of viability. In 1950 the eggs laid by the ninth generation were given to an assistant to be kept during the absence of the senior author. All were dead when examined the following year.

4. Selection for development without diapause (1953-1960)

Eggs hatching without a diapause and which had been laid by a female *Melanoplus d. differentialis* caught near Iowa City in 1953 were used in a second attempt to isolate a stock with no diapause. For six generations the stock was carried on in mass culture with pods containing large numbers of no diapause eggs being saved to produce the next generation. A single pod with 71.1 percent no diapause eggs was used to obtain the seventh generation and a pod with 43.6 percent the eighth. The next two generations were carried on in mass cultures. The grasshoppers hatching from a single pod with 100 percent no diapause eggs laid by the tenth generation were mated in pairs and this was repeated with the grasshoppers hatching from a single pod containing 89.2 percent no diapause eggs in the eleventh generation. The twelfth generation has proven to be extremely weak, and it is not yet certain that the line can be continued. Attempts to save it by outcrossing to a different stock are being made.

The results of the 12 generations of selection are presented in Table VI. The marked decrease in vigor of the stock, as selection is continued, is apparent when the drop in the number of egg pods, the number of eggs per pod and the percentage of viable eggs are noted. Since this did not occur in the 1928-1932 and 1943-1950 series of experiments, it is probable that the loss of viability is due not to the genes concerned with diapause, or its absence, but rather to the accumulation of other genes which are deleterious and which were accidentally included during selection. Although a true-breeding stock without diapause has not been obtained, it is again obvious that the incidence of the character can be markedly increased by selection and that it is under genetic control.

Discussion

Le Berre[2, 3, 4] has investigated the inheritance of diapause in the eggs of several subspecies of *Locusta migratoria* which occur in France and in each of which the eggs develop in a different manner. *Locusta m. cinerascens* produces eggs all of which develop without a diapause. *Locusta m. gallica,* on the other hand, and an unnamed subspecies from Palavas lay eggs most of which enter diapause, while a few complete embryonic development without it. Some of the eggs of the subspecies *gallica* undergo what Le Berre calls an "intermediate diapause" which is temporary and independent of temperature. Le Berre[4] crossed the subspecies *gallica* and *cinerascens* and states that diapause, or the lack of it, is inherited in a complex manner. He found the F_1 to be matroclinous at temperatures of 25°C. and below. Le Berre[3] succeeded in isolating two stocks of *Locusta migratoria gallica,* one of which produced only eggs with diapause and the other only eggs without diapause. The stock without diapause proved to be poorly viable and died out in the ninth generation, while the stock with a diapause was continued throughout the tenth generation.

In comparing the present results with those of Le Berre it must be pointed out that Le Berre defined true diapause eggs as those which will not hatch unless treated with cold or some other agent, such as xylol, to break diapause. The present authors, in contrast, have classed as diapause all eggs which do not hatch on or close to the thirty-eighth day at 25°C. Le Berre's eggs without diapause include all those which hatch without treatment to break diapause. Uninterrupted development in his species requires about 14 days at 33°C. His stock with no diapause in the first generation consisted of eggs which hatched between 14 and 156 days at 33°C. and in the eighth generation was composed of eggs which hatched between 13 and 25 days. The present writers would have excluded all eggs from this category in which development is even briefly interrupted.

Essentially, however, the results of Le Berre and those reported here are in complete agreement. He demonstrated that diapause and its absence are under genetic control and that selection in either direction is effective. He did not suggest the form of inheritance in-

volved beyond saying that it is complex. The authors of the present paper feel that a system of polygenes is probably involved in the control of diapause. Such phenomena as reduced penetrance, variable expressivity, pleiotropy, epistasis, etc., may well play a part and make an exact genetic analysis impossible in the present state of our knowledge. A discussion of the current status of the polygene concept is given by Lerner[6].

In a series of six papers published by the senior author between 1946 and 1958 it has been shown that diapause in the eggs of *Melanoplus differentialis* is initiated shortly before the close of the third week of incubation at 25°C. by the waterproofing of the hydropyle area of the chitinous cuticle. It is through this area that the water required for development enters the egg. The waterproofing material is either a wax or a wax-like substance and is secreted by the cells which lie below the hydropyle. It may be removed by treating the external surface with wax solvents, after which development is resumed providing the egg is supplied with water. After diapause has been broken by cold treatment the waterproof coating of the hydropyle is no longer intact but is speckled with innumerable minute holes which appear to be the ends of pore canals from which the wax has disappeared. Water now enters through these small holes and growth begins. The hydropyle of an egg treated with xylol, in contrast, is uniformly porous over the entire surface and the hydropyle of an egg in diapause is completely waterproofed. A summary of the evidence for the description of diapause just given and references to the earlier literature may be found in the paper published in 1958[15].

Since, then, we have abundant evidence that diapause in the eggs of *Melanoplus differentialis* is under genetic control and that the onset of diapause is immediately preceded by the secretion of waterproofing materials at the surface of the hydropyle, the genes which must be directly concerned are those which determine the capacity of the hydropyle cells for producing these wax-like substances. The hydropyle cells secrete waxy materials not only in those eggs which enter diapause but also in those which do not; so, whether or not diapause ensues must be dependent upon the efficiency of the process. Either more wax-like material is produced by the diapause egg or else it is qualitatively superior as a waterproofing agent. In other words, we have here an instance of inheritance involving continuous variation. The difficulties of analyzing such a situation are great in any case, but with a species such as *Melanoplus differentialis* in which no more than three generations can be obtained in a year progress is very slow.

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